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INVESTIGATING THE POTENTIAL OF HIGH-PRESSURE/HIGH-TEMPERATURE PROCESSING REGARDING TEXTURE PRESERVATION OF PROCESSED FRUITS AND VEGETABLES:

A CASE STUDY ON CARROTS

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VOORWOORD

Na mijn studies tot bio-ingenieur twijfelde ik wat ik zou gaan doen: doctoreren of (zoals soms geformuleerd door anderen) "echt" gaan werken. Toen ik ging informeren bij Prof. Hendrickx, sprak hij vol enthousiasme over een groot Europees project (NovelQ) met alle relevante Europese topwetenschappers dat van start zou gaan. Doctoreren binnen een EU-project zou toch wel een extra dimensie geven. Beetje onwetend, onder de indruk en nieuwsgierig ben ik hem gevolgd in zijn enthousiasme… met nu dit boekje tot gevolg. Dit boekje is echter maar een deeltje van vier jaar doctoreren: de voorbije jaren heb ik, zowel op professioneel als op persoonlijk vlak, ook massa's bijgeleerd en beleefd. Voor dit alles wil ik vele mensen bedanken.

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Ans

SAMENVATTING

Het thermisch behandelen van fruit, groenten en hun afgeleide producten met het oog op conservering gaat gepaard met aanzienlijke nutritionele en sensorische kwaliteitsverliezen. Textuurverlies treedt op en is, naast verlies van turgordruk, hoofdzakelijk te wijten aan chemische veranderingen in de pectine-celwandpolysachariden. Hoge druk (HP)-procesvoering is opgekomen als een alternatieve procestechnologie die een beter evenwicht tussen voedselveiligheid en -kwaliteit garandeert voor diverse producten. Momenteel is HP-procesvoering gelimiteerd tot gekoelde of milde temperaturen en resulteert in gepasteuriseerde levensmiddelen. De combinatie van HP en hoge temperatuur (HT) heeft bewezen te resulteren in gesteriliseerde levensmiddelen. Studies betreffende het effect van HP/HT-procesvoering op voedselkwaliteit zijn echter schaars. Naast conserveringsdoeleinden kan HP ook aangewend worden om nieuwe functionaliteiten te creëren door specifieke wijzigingen te induceren. Deze toepassing vereist echter een gedetailleerde kennis (op moleculair niveau) van de effecten van HP op biologische materialen. De voorliggende studie onderzoekt in deze context het potentieel van HP/HT-procesvoering aangaande het textuurbehoud van verwerkte groenten en fruit.

In een eerste, verkennend deel werd het effect van HP in combinatie met licht verhoogde temperatuur op de textuur van wortelen en op de onderliggende celwandchemie onderzocht. Wortelen werden onderworpen aan drie verschillende behandelingen (80 °C/0,1 MPa, 100 °C/0,1 MPa en 80 °C/600 MPa). Vervolgens werden de residuele hardheid en microstructurele veranderingen geëvalueerd. Het celwandmateriaal werd geïsoleerd uit de wortelstalen en de pectinestructuur geanalyseerd. Thermische behandelingen bij 0,1 MPa veroorzaakten een uitgesproken weefselverzachting. Dit ging vergezeld van een opmerkelijke celscheiding, een toename in het gehalte aan waterextraheerbaar pectine (WSP) en een overeenkomstige afname in het gehalte aan pectine extraheerbaar met een oplossing van een chelerend agens (CSP) en een natriumcarbonaatoplossing (NSP). HP/HT-behandelde wortelen vertoonden daarentegen minimale weefselverzachting en verwaarloosbare veranderingen in intercellulaire adhesie. Op moleculair niveau werd een opmerkelijke reductie in de veresteringsgraad (DM) van pectine waargenomen, evenals een laag gehalte WSP in tegenstelling tot hoge gehaltes CSP en NSP, beperkte veranderingen in de verschillende pectinefracties tijdens behandeling en een substantiële hoeveelheid pectine in het fractionatie-residu. De resultaten duidden duidelijk op een verschillend gedrag van wortelen wanneer een thermisch proces gecombineerd werd met verhoogde druk.

De beta-eliminatieve depolymerisatie van pectine wordt beschouwd als één van de hoofdoorzaken van het thermisch verzachten van laag-zure groenten en fruit. De reactiesnelheid is sterk afhankelijk van de DM van pectine en het verlagen van de DM heeft bewezen het textuurverlies te reduceren. Het effect van HP/HT-procesvoering op de beta-eliminatiereactie en de chemische ontestering werd onderzocht in modelsystemen op basis van pectine. Pectine-oplossingen bij pH 6,5 werden onderworpen aan HT (70 – 120 °C) bij 0,1 MPa en aan HT bij verhoogde druk (90, 110 en 115 °C / 500, 600 en 700 MPa). Vervolgens werd de mate van beta-eliminatie en ontestering bepaald. Bij 0,1 MPa namen de nulde-orde reactiesnelheidsconstanten van beide reacties toe met toenemende temperatuur. Bij alle temperaturen vertoonde ontestering een hogere snelheidsconstante dan beta-eliminatie. Een temperatuursverhoging resulteerde echter in een sterkere toename van beta-eliminatie dan van ontestering. Door het combineren van verhoogde temperatuur en HP werd de beta-eliminatie vertraagd of gestopt terwijl de ontestering sterk werd gestimuleerd.

De textuurdegradatie van wortelen tijdens thermische en HP/HT-behandelingen werd in detail bestudeerd. Wortelen werden thermisch (0,1 MPa) of HP/HT (600 MPa) behandeld in een temperatuurszone van 95 tot 110 °C. De evolutie van de hardheid kon goed beschreven worden door een model voor fractionele conversie. In vergelijking met de thermische behandelingen resulteerden de HP/HT-behandelingen in een tienmaal tragere textuurdegradatie en een celwandpectine met een opmerkelijk lagere DM.
Tevens werd het effect van het verlagen van de DM (door het toepassen van een HP-voorbehandeling) en het toevoegen van exogene calciumionen (door drenking in een Ca^{2+}-oplossing), voorafgaand aan de HP/HT-behandeling, op de textuur van wortelen onderzocht. Er werd vastgesteld dat deze gecombineerde voorbehandeling resulteerde in een opmerkelijk hardere textuur van HP/HT-behandelde wortelen. Een gelijkaardig resultaat werd echter bekomen door wortelen rechtstreeks HP/HT te behandelen in een calciumchloride-oplossing, zonder voorafgaandelijke DM-reductie en drenking in een Ca^{2+}-oplossing.

Equivalente thermische en HP-processen, van zowel pasteurisatie- als sterilisatie-intensiteit, werden geëvalueerd wat betreft hun effect op de hardheid en microstructuur van wortelen. Voor beide procesintensiteiten vertoonde HP-procesvoering een beter behoud van textuur dan thermische procesvoering. Thermisch gepasteuriseerd wortelweefsel verloor 80% van zijn initiële hardheid en vertoonde celscheiding. In geval van HP-pasteurisatie beperkte het hardheidsverlies zich tot 40% en werd een goede celadhesie vastgesteld. Zowel het thermische als het HP-sterilisatieproces werden gekarakteriseerd door verregaande weefselverzachting: respectievelijk 2 en 12% van de initiële hardheid

werden slechts behouden. Deze extensieve textuurdegradatie kwam ook tot uiting in de wortelmicrostructuur, waar intense celscheiding en polymeersolubilisatie werden waargenomen, het meest uitgesproken in geval van thermische sterilisatie.

Er kan besloten worden dat HP/HT-procesvoering resulteert in een beter textuurbehoud van wortelen dan thermische procesvoering bij atmosfeerdruk. De verhoogde druk leidt tot kortere procestijden en een toegenomen ontestering van pectine.

ABSTRACT

Thermal processing for preservation of plant derived food products entails considerable nutritional and sensory quality losses. Texture loss of fruits and vegetables occurs, next to turgor pressure loss, mainly due to chemical changes in the cell wall pectic polysaccharides. High-pressure processing (HPP) has emerged as a strong alternative food processing technology enabling a better balance between safety and quality for various products. Currently, HPP is limited to chilled or mild temperatures and results in pasteurized food products. The combination of high-pressure (HP) and high-temperature (HT) has proven to result in sterilized food products. However, studies on the effect of HP/HT processing on food quality are rather scarce. In addition to preservation purposes, HP can be applied to create novel functionalities by inducing specific changes. This application requires a detailed understanding of the effects of pressure on biological materials at the molecular level. In this context, this study examines the potential of combined HP/HT processing regarding texture preservation of processed fruits and vegetables.

In a first, exploratory part, the effect of combining HP with slightly elevated temperature on the texture of carrots and the underlying cell wall chemistry was investigated. Carrots were subjected to three different treatments (80 °C/0.1 MPa, 100 °C/0.1 MPa, 80 °C/600 MPa). Subsequently, the residual hardness and microstructural changes were evaluated. The cell wall material was isolated from the samples and the pectin structure analysed. Thermal treatments at 0.1 MPa caused extensive tissue softening. This was marked by increased cell separation, an increase in water soluble pectin (WSP) paralleled by a decrease in chelator (CSP) and sodium carbonate (NSP) soluble pectin. HP/HT treated carrots showed minimal softening and negligible changes in intercellular adhesion. This was accompanied by a significant reduction in the pectin degree of methoxylation (DM), low WSP in contrast to the high CSP and NSP fractions, minor changes in the different pectin fractions during treatment, and a substantial amount of pectin in the fractionation residue. The results clearly indicated different behaviour of carrots when a thermal process was combined with elevated pressure.

The beta-eliminative depolymerisation of pectin is one of the main causes of thermal softening of low-acid fruits and vegetables. The reaction rate is strongly dependent on the pectin DM and lowering the DM has proven a strategy to reduce texture loss. Therefore, the effect of HP/HT processing on the beta-elimination reaction and chemical demethoxylation was assayed in pectin model

systems. Pectin solutions at pH 6.5 were subjected to HT (70 – 120 °C) at 0.1 MPa and to HT at elevated pressure (90, 110 and 115 °C / 500, 600 and 700 MPa). Subsequently, the extent of beta-elimination and demethoxylation was determined. At 0.1 MPa, zero-order reaction rate constants for both reactions increased with increasing temperature. At all temperatures, demethoxylation showed a higher rate constant than beta-elimination. However, a temperature rise resulted in a stronger acceleration of beta-elimination than of demethoxylation. When combining elevated temperature and HP, beta-elimination was retarded or even stopped, whereas demethoxylation was strongly stimulated.

Texture degradation kinetics of carrots during thermal and HP/HT processing were studied in detail. Carrots were thermally (0.1 MPa) or HP/HT (600 MPa) treated in a temperature range from 95 to 110 °C. Hardness evolution could be well described by a fractional-conversion model. Compared to thermal treatments, the HP/HT treatments resulted in a tenfold slower texture degradation. The retarded texture loss was accompanied by a significantly lower DM of the cell wall pectin.
Additionally, the effect of lowering the DM (by applying a HP pretreatment) and adding exogenous Ca^{2+} (by soaking in a Ca^{2+} solution) prior to the HP/HT treatment on the texture of carrots was investigated. It was observed that this combined pretreatment resulted in a notably harder texture of HP/HT treated carrots. However, a similar outcome was obtained by HP/HT treating carrots directly in a calcium chloride solution without preceding DM reduction and Ca^{2+} soak.

Equivalent thermal and HP processes, both pasteurization and sterilization intensities, were evaluated regarding their effect on carrot hardness and microstructure. For both processing intensities, HPP proved to be preferred to thermal processing. Thermally pasteurized carrot tissue lost 80% of its initial hardness and displayed cell separation. In case of HP pasteurization, hardness loss was limited to 40% and good cell adhesion was observed. Both thermal and HP sterilization processes were characterized by extensive tissue softening: respectively 2 and 12% of the initial hardness remained. This large texture degradation was reflected in the carrot microstructure where extensive cell wall separation and polymer solubilisation was observed, the most outspoken in case of thermal sterilization.

It can be concluded that HP/HT processing results in a better texture preservation of carrots than thermal processing at 0.1 MPa. The elevated pressure entails reduced process times and enhanced pectin demethoxylation.

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LIST OF NOTATIONS

List of symbols

p	pressure
t	time
T	temperature

List of abbreviations

AIR	alcohol insoluble residue
Ara	arabinose
C.	*Clostridium*
CDTA	cyclohexane-trans-1,2-diamine tetra-acetic acid
CSP	chelator soluble pectin
DM	degree of methoxylation
Gal	galactose
GalA	galacturonic acid
HG	homogalacturonan
HP	high-pressure
HP/HT	high-pressure/high-temperature
HPP	high-pressure processing
HT	high-temperature
MES	2-(N-morpholino)ethanesulfonic acid
MM	molecular mass
NS	neutral sugar
NSP	sodium carbonate soluble pectin
PME	pectin methylesterase
POM	polyoxymethylene acetal
RG-I	rhamnogalacturonan I
RG-II	rhamnogalacturonan II
Rha	rhamnose
TRIS	2-amino-2-hydroxymethyl-1,3-propanediol
WSP	water soluble pectin

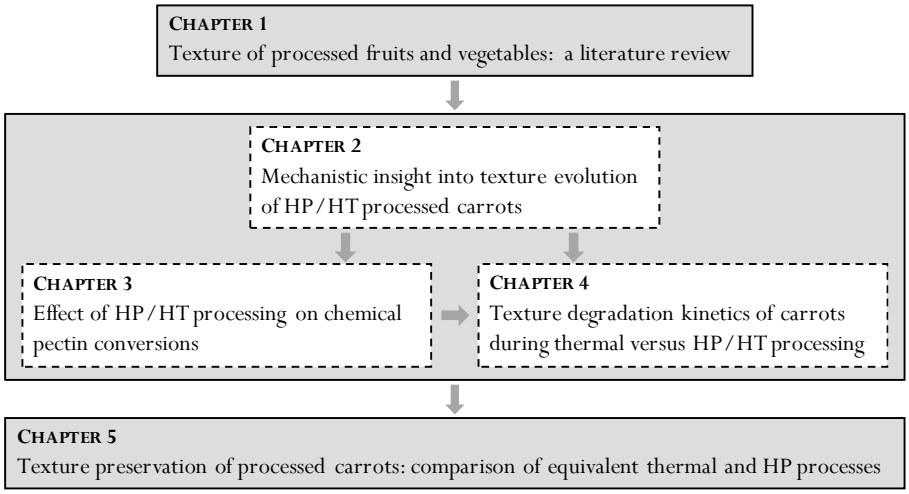
GENERAL INTRODUCTION

To prolong the shelf life of food products, processing is often necessary. Traditional heating methods for preservation of packed foods have the drawback of a slow heating and cooling rate, extending the total treatment time, adversely affecting nutritional and sensory quality characteristics. Therefore, food scientists and the food industry are in quest of technologies that destroy undesired micro-organisms and enzymes but retain product quality better. High-pressure processing (HPP) has been proven a worthy alternative. In current commercial applications, HPP is an essentially 'non-thermal' pasteurization process, in which a food is subjected to pressures up to 600 MPa and initial temperatures lower than 45 °C for one to twenty minutes. Since the process involves minimal heating, the nutritional and sensory qualities of pressure-treated products can be remarkably similar to those of their unprocessed counterpart. Unfortunately, bacterial spores are very resistant at commercially achievable pressure levels. Therefore, high-pressure (HP) processed food products currently on the market are chilled and/or high-acid (e.g. fruit juices, guacamole, ham). To obtain ambient stable low-acid foods, an additional factor causing inactivation is required. In HP sterilization, this factor is elevated temperature. By starting the HP treatment at temperatures between 60 and 90 °C and by using the compression heat for rapid and uniform heating to higher temperature levels, sterilization conditions can be achieved. The main potential benefit is the instantaneous and volumetric heating and cooling, which - in theory - could lead to reduced processing times and improved food quality. To date, data published on the effect of HP sterilization on food quality attributes are rather sparse.
Texture is a major quality parameter of fruits and vegetables and significantly changes during a thermal process. The underlying plant structure is a key determinant of plant texture. Beta-eliminative depolymerisation of the cell wall pectic polysaccharides has (partly) been accounted for the textural damage during thermal processing. By solubilising pectin, this depolymerisation results in decreased intercellular adhesion and consequently in tissue softening. Texture improvement of thermally processed fruits and vegetables has been approached in various ways. An established strategy is to render the plant tissue less vulnerable to the beta-elimination reaction by lowering the pectin degree of methoxylation. Enhancement of cell adhesion by increasing the number of pectin cross-links in the cell wall is another (additional) approach.

The objective of this work (in Scheme 1 an outline of the work is shown) is to investigate what level of texture improvement of fruits and vegetables, as compared to thermal processing at 0.1 MPa, can be achieved by combining high-

temperature (HT) and HP. By influencing rate constants of key pectin changes, this additional process variable might lead to different product characteristics as compared to heat. Carrots were selected for the study to obtain generic insight as they have a high pectin content and their textural changes largely depend on pectin modifications.

Chapter 1 comprises a literature review on texture of processed fruits and vegetables. First, both thermal and HP processing are discussed in general. Afterwards, the quality parameter texture and how this parameter is influenced by processing are reviewed. In a first experimental chapter (Chapter 2), the effect of HPP in combination with slightly elevated temperature on the texture of carrots is explored. Mechanistic insight is obtained by examining different levels of plant structure. In subsequent chapters, processing temperatures are more severe, i.e. approximating HP sterilization conditions. A more detailed understanding of the effects of pressure at the molecular level is gained in chapter 3 by investigating chemical pectin conversions (beta-eliminative depolymerisation and chemical demethoxylation) in pectin model systems. In chapter 4, the texture degradation kinetics of carrots during thermal and HP/HT processing are studied in detail. In addition, several approaches for texture improvement are evaluated. The final chapter (Chapter 5) is more practically oriented and aims at validating the previous findings at industrially applied processing conditions. Equivalent thermal and HP processes (both pasteurization and sterilization intensities) are compared regarding their effect on hardness and microstructure of carrots.



Scheme 1: Outline of the present thesis.

1 TEXTURE OF PROCESSED FRUITS AND VEGETABLES: A LITERATURE REVIEW

1.1 FOOD PRESERVATION

Since ancient times, man has developed different ways to preserve food (e.g. drying, freezing, cooling, heating). Food preservation allows a time span between production and consumption. Until now, thermal processing has been the most commonly used preservation technology since it allows efficient inactivation of both pathogenic and spoilage micro-organisms as well as enzymes with deteriorative action. Unfortunately, thermal processing is also accompanied by nutritional and sensory quality losses. Nowadays, consumer preferences point toward products that, while having an extended shelf life, have characteristics similar to the "fresh", unprocessed product. This demand has incited the food industry to continuously improve the existing technologies and has given birth to many research efforts on novel technologies. Over the years, thermal processing has been optimized for producing better quality foods by introducing several approaches which result in reduced process times. New technologies such as high-pressure processing (HPP) and pulsed electric field processing have emerged as non-thermal alternatives. These new technologies are in an advanced state of maturity (both in terms of their food science basis and their technology development) and have recently been introduced or are likely to be introduced in the near future at an industrial scale.

This chapter starts with a brief overview of traditional thermal processing. Both the desired and undesired effects of applying heat on food quality are highlighted, as well as technological implementations to diminish the undesired quality losses. In a second part, HPP is introduced; basic principles, technical aspects, effects on quality related food attributes and current applications are successively discussed. Finally, the current main challenge of the technology, i.e. the combined use of high-pressure (HP) and high-temperature (HT) (e.g. commercial HP sterilization) is outlined. The main focus is on fruit and vegetable products.

1.1.1 THERMAL PROCESSING

1.1.1.1 Thermal process considerations

Thermal processing for food preservation involves heating of a food for a set time at a set temperature to destroy pathogenic and/or spoilage micro-organisms and to inactivate enzymes with deteriorative action. Based on the severity of the thermal process, distinction can be made between pasteurization and sterilization (Ramaswamy and Marcotte, 2006a). Pasteurization is a mild heat treatment (usually temperatures below 100 °C) intended to destroy selected vegetative microbial species (particularly the pathogens). As the process does not inactivate all vegetative micro-organisms and hardly affects spores, pasteurized foods have a rather limited shelf life (from several days to several months) and need to be stored under conditions which minimize microbial growth and prevent spore germination (e.g. refrigeration, low pH). Sterilization is a more severe heat treatment (temperatures exceeding 100 °C, mostly 120 – 140 °C) intended to destroy both vegetative micro-organisms and spores, resulting in shelf stable foods. Actually, a sterilization process is not designed to destroy all micro-organisms present in a food. Such a process would result in low product quality due to the long heating required. The success of thermal processing rather depends on destroying all pathogenic and most spoilage micro-organisms while creating an environment that does not support the growth of the more resistant thermophilic bacteria not fully destroyed by the heat treatment (Ramaswamy, 2005; Ramaswamy and Marcotte, 2006a; Ganzle *et al.*, 2007). Important factors in determining the required intensity of the thermal process are the heat resistance of the target micro-organism, spore, or enzyme present in the food, the pH of the food, and the storage conditions following the process.
From a public health standpoint, the most important micro-organism in low-acid (pH > 4.6) foods is *Clostridium botulinum*, a heat-resistant, spore-forming, anaerobic pathogen, which can produce the deadly botulism toxin (Ramaswamy, 2005; Ramaswamy and Marcotte, 2006a; Ganzle *et al.*, 2007). It has been generally recognized that *C. botulinum* spores do not germinate and grow in foods below a pH of 4.6. Hence, in acid foods *C. botulinum* spores may be present and relatively mild heat treatments can be applied to destroy the vegetative cells only (pasteurization process). In low-acid foods, relatively severe thermal treatments have to be applied in order to kill the *C. botulinum* spores. To produce low-acid ambient stable products, standard industrial practice is to apply, as a minimum, a thermal process equivalent to 121.1 °C for 3 min at the slowest heating point of the product. This process is designed to deliver at least a 12 log reduction of proteolytic *C. botulinum* spores (Ganzle *et al.*, 2007; Leadley *et al.*, 2008).

1.1.1.2 Technical aspects of thermal processing

The technological implementation of thermal processing can be subdivided in in-pack and aseptic processing (Ramaswamy, 2005; Ramaswamy and Marcotte, 2006a). In-pack processing consists of filling product into containers (metal, glass or plastic), which are sealed and processed in some type of batch or continuous retort system (May, 2001; Bown, 2001). The quest for improved food quality has resulted in the development of aseptic processing, which involves heating and cooling a liquid product (with or without particulates), and then filling into sterile packages and sealing under aseptic conditions (Emond, 2001). Heating of unpacked liquid foods is most commonly accomplished in continuous heat exchangers. Due to rapid heating and cooling, the latter process generally yields higher quality products (see section 1.1.1.3).

1.1.1.3 Thermal processing and food quality

Thermal processing affects, next to micro-organisms and enzymes, also nutritional and sensory food quality attributes (such as appearance, texture and flavour). The extent of modification depends on the intensity of the thermal process (Ramaswamy, 2005). Nutritional and sensory characteristics of most foods are only slightly influenced by a pasteurization process as opposed to a sterilization process which can result in serious quality degradation.

One of the major problems with conventional in-pack thermal processing is the slow heat transfer rate (Ramaswamy *et al.*, 2005). Heat penetration to the slowest heating point in the container happens by means of conduction and convection (Ramaswamy and Marcotte, 2006b). By the time the required degree of lethality is achieved in the centre of the product, the product at the container surface very often gets over-processed, resulting in great nutritional and sensory quality destruction. To improve the overall rate of heat transfer to a packaged food, different approaches have been introduced, such as container agitation and thin profile processing (Ramaswamy *et al.*, 2005; Ramaswamy and Marcotte, 2006a). By respectively agitating the packaged product or reducing the package thickness, the required amount of heat to be applied to the critical point is transferred faster with minimal over-processing of the product near the container surface. The observation that the temperature dependence of quality degradation is much lower than that of microbial inactivation has led to the high-temperature short-time processing techniques. Next to allowing a shorter process time (and thus higher throughput), the application of high-temperatures can promote better food quality. However, these techniques are based on the assumption that the temperature distribution inside the food during processing is uniform and rapidly changing. For liquid foods, especially for those with low viscosity such as milk and fruit beverages, this assumption can be easily realized with the application of various types of heat exchangers. However, for solid foods, particularly those

stored in larger containers, neither rapid change nor uniformity is easy to achieve (Ramaswamy *et al.*, 2005; Ramaswamy and Marcotte, 2006a). The overall problem of slow heat transfer can be circumvented by using volumetric heating techniques, such as microwave, radio-frequency and ohmic heating (Ohlsson, 1999). By interaction with an external electromagnetic field, heat is directly generated inside the food.

1.1.2 HIGH-PRESSURE PROCESSING

1.1.2.1 Introduction

Currently HPP, also referred to as high-hydrostatic-pressure or ultra-high-pressure processing, uses pressures up to 600 MPa at chilled or mild process temperatures (< 45 °C) to inactivate vegetative micro-organisms and spoilage enzymes present in food, while minimally affecting sensory and nutritional quality attributes (Balasubramaniam *et al.*, 2008). Unfortunately, bacterial spores are very pressure resistant. Combination treatments using HP and heat have been proposed as a method for producing shelf stable low-acid foods (Meyer *et al.*, 2000). Although the principles of HPP for microbial inactivation have been known since the late 1800s (Hite, 1899), it was not until the early 1990s that the first commercial food applications of the technology were seen. In addition to food preservation, HPP opens up the possibility of producing foods with novel texture (e.g. meat, fish, dairy products) by modifying functional properties of food constituents such as proteins (Cheftel, 1992; Heinz *et al.*, 2009).
In the following sections, HPP is discussed in the context of its current main commercial application, i.e. a non-thermal pasteurization process; thus assuming chilled or mild process temperatures. Only the last section (1.1.2.6) is devoted to the combined use of HP and HT with the aim of HP sterilization.

1.1.2.2 Basic principles of high-pressure processing

Two general scientific principles govern the behaviour of foods under pressure: the principle of Le Chatelier and the principle of Pascal (Cheftel, 1992; Yuste *et al.*, 2001). The principle of Le Chatelier indicates that any phenomenon (phase transition, change in molecular conformation, chemical reaction) accompanied by a decrease in volume is enhanced by an increase in pressure, and vice versa. Thus, interactions among biomolecules (i.e. covalent and hydrogen bonds, electrostatic and hydrophobic interactions) are modified in one way or another depending on whether the formation of these interactions results in a positive or negative volume change. Contrary to thermal treatments, where covalent as well as non-covalent bonds are affected, HPP at room temperature only disrupts non-covalent bonds (Hendrickx *et al.*, 1998). According to the Pascal or isostatic

principle, pressure is transmitted uniformly and instantaneously throughout the entire sample. This makes HPP independent of the size and shape of the sample and also shortens processing time. The product does not become deformed despite being under such high pressures, providing the food does not have a porous structure containing air voids (such as in strawberries). Air pockets collapse under pressure due to differences between the compressibility of air and water, and the food is not restored to its original size and shape (Balasubramaniam *et al.*, 2008).

1.1.2.3 Technical aspects of high-pressure processing

The primary components of a HPP system include a pressure vessel, a pressure-transmitting medium, a temperature-control device, and a pressure-generation system (Mertens and Deplace, 1993; Mertens, 1995; Patterson, 2005). The pressure vessel is, in many cases, simply a forged monolithic, cylindrical vessel constructed of low-alloy steel of high tensile strength. The wall thickness is determined by the maximum working pressure, the internal diameter of the vessel, and the number of cycles for which the vessel is designed. The required wall thickness can be reduced by using multilayer, wire-wound, or other pre-stressed designs. Temperature control is mostly achieved by circulating a heating/cooling medium through a jacket surrounding the pressure vessel. However, this type of control yields poor heating/cooling rates. When faster temperature changes are required, an internal heat exchanger is fitted. A conventional batch HP process uses food products (liquids and/or solids) packed in a flexible container. These packages are loaded into the vessel, which is closed and filled with a pressure-transmitting medium. Typically, water mixed with a small percentage of soluble oil for pump lubrication is used. The system is pressurized to its operating pressure either by direct or indirect compression. In the case of direct compression, the system is directly pressurized by a piston. Pressurization by means of indirect compression takes place through an external HP intensifier which pumps pressure medium into the vessel. Once the desired pressure is reached, pumping is stopped, valves are closed and the pressure can be maintained without further need for energy input. The pressure is released after the desired treatment time (usually between 30 seconds and 15 minutes) and the food packages are unloaded. Next to batch systems, semi-continuous systems exist which can be applied to pumpable products only (e.g. juices) (Heinz *et al.*, 2009). The product is pumped in the pressure vessel and pressurized using a floating piston, which separates the product from the pressure-transmitting medium. Several pressure vessels can be served in parallel by the same generator, so that a continuous downstream flow can be maintained.

Pressure vessels used for commercial food production have internal volumes ranging from 30 to more than 600 litres and both horizontal and vertical vessel

configurations are available. Commercial-scale, HPP systems cost approximately 0.5 to 2 million euros, depending on equipment capacity and extent of automation (Balasubramaniam *et al.*, 2008). Although the capital equipment cost is relatively high, processing costs are relatively low (Patterson, 2005). Depending on the operating parameters and the scale of operation, the cost of HP treatment is typically around 0.1 – 0.2 euros per litre whereas the cost for thermal treatment may be as low as 0.02 – 0.04 euros per litre. Avure Technologies (USA), NC Hyperbaric (Spain), and Uhde (Germany) are major suppliers of commercial-scale HP equipment for food processing (Balasubramaniam *et al.*, 2008).

1.1.2.4 Effects of high-pressure on quality related food attributes

Micro-organisms

The lethal effect of HP on vegetative micro-organisms is thought to be the result of a number of different processes taking place simultaneously, in particular damage to the cell membrane and inactivation of key enzymes, including those involved in DNA replication and transcription (Smelt, 1998; Yuste *et al.*, 2001; Patterson, 2005).

Micro-organisms vary in their response to pressure (Patterson, 2005). Yeasts and moulds are most pressure-sensitive and inactivated by pressures between 200 and 300 MPa. Gram-negative bacteria can be inactivated by pressures of about 300 MPa and are, in their turn, less pressure-stable than gram-positive bacteria, for which pressures higher than 400 MPa are required for inactivation. However, numerous exceptions to these general statements can be found. Bacterial spores can be extremely resistant to HP and can survive treatments of more than 1000 MPa at ambient temperature (Smelt, 1998). However, relatively low pressures (around 300 MPa) can trigger spore germination. This has led to the suggestion that spores could be killed by applying pressure in two stages. The first pressure treatment would induce spore germination while the second treatment, at a higher pressure, would kill the germinated spores. However, the extent of inactivation can be highly variable. Another approach is to combine HP with elevated temperature. Currently, this approach is being actively considered for the commercial production of shelf stable low-acid foods (see section 1.1.2.6). Although the terms "pasteurization" and "sterilization" were originally introduced to specify the destruction of micro-organisms by heat, their use has been extended to HPP.

Next to the type of micro-organism, other factors determining the extent and rate of inactivation are pressure level, treatment time, temperature, food composition, pH, and water activity (Yuste *et al.*, 2001; Patterson, 2005). The pressure resistance of micro-organisms often reaches a maximum at ambient temperature, so the inactivation can be improved by reducing or elevating the

temperature of the food prior to HPP. Many food components (proteins, carbohydrates, lipids) protect micro-organisms from the effects of pressure treatment and it will often take a longer processing time or higher pressure level to achieve the same degree of inactivation in food versus buffers or laboratory media. Acidification or antimicrobial agents work generally synergistically with pressure in destroying micro-organisms. Reduced water activity, however, tends to inhibit pressure inactivation with noticeable retardation as the water activity falls below about 0.95.

Food quality related enzymes

Enzymes are a special class of proteins in which biological activity arises from an active site, brought together by the three-dimensional structure of the molecule. HP may affect enzymes in several manners (Cheftel, 1992). (i) Pressurization may bring about reversible or irreversible, partial or complete enzyme inactivation resulting from conformational changes in the protein structure. These changes depend on the type of enzyme, pressure level, temperature and processing time. (ii) An enzymatic reaction may be enhanced or retarded by pressure, depending on the positive or negative reaction volume. (iii) A macromolecular substrate may become more sensitive to enzymatic modification. (iv) Provided that the cell membrane or the membranes of intracellular organelles are altered by pressure, intracellular enzymes may be released in the extracellular fluids or the cell cytoplasm, hereby facilitating enzyme-substrate interactions.

With respect to enzymes related to quality of fruit and vegetable products, commodities for which HP treatment is believed to offer great potential in the area of preservation and processing, many research efforts have been focused on enzyme inactivation (Ludikhuyze *et al.*, 2001). Key enzymes in the fruit and vegetable processing industry are polyphenol oxidase, lipoxygenase, pectin methylesterase (PME) and peroxidase. Thermal inactivation kinetics of these enzymes have been studied extensively in the past and are generally well documented. Throughout the last decades the number of studies on pressure inactivation have consistently been increasing (Hendrickx *et al.*, 1998; Indrawati *et al.*, 2003). Some enzymes can be readily inactivated at room temperature by a few hundred MPa, while others can withstand 1000 MPa for prolonged treatment times. Because of the extreme pressure stability of some enzymes, combined processes (e.g. pressure and temperature) might be necessary for inactivation at industrially relevant pressures.

Sensory and nutritional food attributes

Important characteristics of food quality are texture, colour, flavour and nutritional value. As HP at room temperature keeps covalent bonds intact, it follows that low-molecular-weight food components such as pigments (e.g. chlorophyll, carotenoids, anthocyanins, etc.), flavour compounds and vitamins

are largely preserved. This leads to the general acceptance that HPP only minimally affects food quality (Ludikhuyze *et al.*, 2001). However, HPP can influence (bio)chemical reactions that can bring about undesirable changes in these quality attributes (Oey *et al.*, 2008a; Oey *et al.*, 2008b). Moreover, during subsequent storage, quality changes can occur due to incomplete inactivation of enzymes and/or micro-organisms. Consequently, the effect of HP on sensory and nutritional properties is not that straightforward and strongly product dependent. Same applies to texture of fruits and vegetables; both firming and softening have been observed (Basak and Ramaswamy, 1998) (see section 1.2.2.3).

1.1.2.5 Commercial applications

Since the early 1990s, HP pasteurized food products can be found on the market (Ludikhuyze *et al.*, 2001; Indrawati *et al.*, 2003; Lau and Turek, 2007). Japan was the first country to introduce HP treated food products, namely fruit jams and sauces. HP treated fruit juice in France, a delicatessen style ham in Spain, and avocado-based products in the United States followed. Nowadays, HPP is applied to fruits and vegetables, meat products, seafood, juices and beverages, and dairy products (Figure 1.1). The use is not limited to preservation purposes only. Product design by pressure-induced structural changes is another emerging field of application (Heinz *et al.*, 2009).

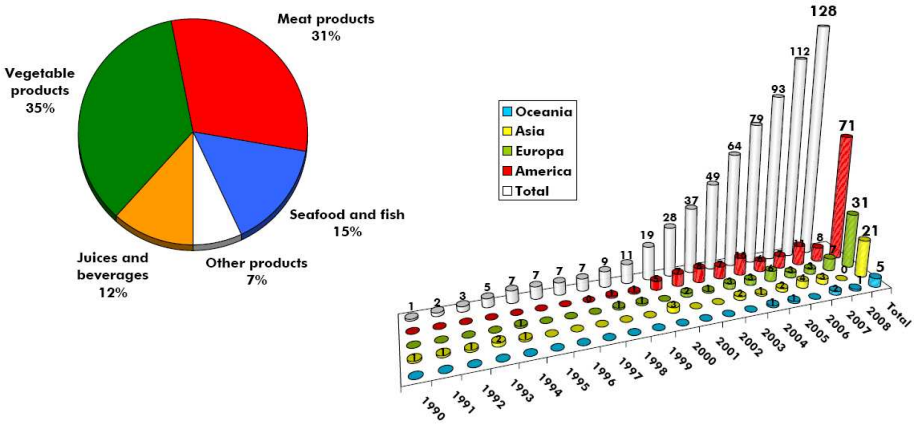


Figure 1.1: Number of industrial HPP machines versus food industries and evolution of industrial HPP machines in the world (NC Hyperbaric, Spain).

By 2008, approximately 130 industrial HPP installations were in use worldwide for commercial-scale food production (Figure 1.1). More than 80% of the HP equipment was installed after 2000, indicating an accelerated trend in HPP use. This has mainly been attributed to a reduction in equipment costs and an

increasing consumer demand for fresher-tasting foods containing fewer preservatives. North America (USA, Canada, and Mexico), Europe (Spain, Italy, Portugal, France, UK, and Germany), Australia, and Asia (Japan, China, and South Korea) lead the commercialization of HP technology (Balasubramaniam *et al.*, 2008).

HPP is unlikely to replace conventional thermal processing because the latter is a relatively cheap, efficient and well-established food preservation method. However, the technology is expected to complement conventional heating and find niche applications for which the higher HPP cost is justified by superior or unique product properties (Mertens, 1995; Heinz *et al.*, 2009).

Whether or not HPP will become widespread in the food industry also largely depends on consumers' acceptance. Research done on consumer perception of emerging technologies has indicated that HPP is rated positively and preferred by consumers over genetic modification, irradiation and other non-thermal technologies (Cardello *et al.*, 2007). Nielsen *et al.* (2009) carried out a qualitative study on consumer attitudes towards HPP of food. Naturalness, improved taste and high nutritional value of the product and environmental friendliness of the process were perceived as the main advantages. On the other hand, they were concerned about their body and health, the higher price of the products, the lack of information about HPP and were sceptical in general. Overall, consumers see potential in HP processed products.

1.1.2.6 High-pressure sterilization

Introduction

HPP, as commercially defined today, is unable to produce shelf stable low-acid products since bacterial spore inactivation requires pressures of at least 800 – 1700 MPa at room temperature, far in excess of what is commercially feasible (Barbosa-Canovas and Juliano, 2008). Several approaches have been described to attain commercial sterility in low-acid foods (De Heij *et al.*, 2003; Ganzle *et al.*, 2007; Barbosa-Canovas and Juliano, 2008). One approach consists of a low-pressure (around 300 MPa) treatment for spore germination, followed by a HP or thermal treatment for germinated spore inactivation (Heinz and Knorr, 2002). However, the extent of germination can be highly variable making this option unlikely for a commercial process (Wilson *et al.*, 2008). Another approach involves preheating the packaged food and applying one (Wilson and Baker, 2000) or more (Meyer, 2000) HP pulses. From an economical perspective, however, a multiple pulse approach is not recommended, as additional cycles decrease equipment lifetime and increase maintenance costs. Hence, preheating the packaged food and applying a single pressure pulse appears a cost-effective approach, which is now being actively considered for the commercial production of shelf stable low-acid foods.

HP/HT processing, also referred to as pressure-assisted thermal processing, involves the use of initial temperatures between 60 and 90 °C in which, through internal compression heating at pressures of 600 MPa or higher, process temperatures can reach 90 to 130 °C (Barbosa-Canovas and Juliano, 2008). Success of the approach depends on the efficient use of compression heat in achieving nearly adiabatic conditions (Barbosa-Canovas and Juliano, 2008). The main potential benefit of HP/HT processing is that compression heating to higher temperatures is instantaneously achieved throughout the entire package volume and does not rely on conduction or convection to heat the centre of the food. Equally, at the removal of pressure, cooling is very fast. This could, in theory, lead to reduced process times and enhanced food quality (Leadley *et al.*, 2008). Moreover, as an additional variable (elevated pressure) is present (compared to thermal processing at 0.1 MPa), quality related food attributes might be affected differently.

Compression heating in HP/HT processing

All compressible substances change temperature during physical compression, an unavoidable thermodynamic effect (Ting *et al.*, 2002; Barbosa-Canovas and Rodriguez, 2005). The magnitude of this temperature change is determined by the initial product temperature, the applied pressure and the material properties of the product by the following equation:

$$\frac{dT}{dp} = \frac{T\alpha}{\rho C_p}$$

where α is the volumetric expansion coefficient (K^{-1}), ρ the density ($kg \cdot m^{-3}$), C_p the specific heat ($J \cdot kg^{-1} \cdot K^{-1}$), T the temperature (K) and p the pressure (Pa). For most food products the material properties are not known, especially because they are a function of pressure and temperature. However, for water, some oils and alcohols, these properties have been published (Ting *et al.*, 2002; Rasanayagam *et al.*, 2003). Depending on the nature of the product and the initial product temperature, the temperature increase may vary from 3 to 9 °C/100 MPa, the lower value applies to water, the higher to oils and alcohols. Since water is a main ingredient in most foods, the compression of most foods exhibits temperature changes very similar to that of water (Table 1.1).

The maximum change in temperature occurs under adiabatic conditions. In practice, pressurization is not adiabatic, since heat is exchanged between the food, the pressure-transmitting medium and the wall of the pressure vessel (De Heij *et al.*, 2002; De Heij *et al.*, 2003). During compression, the food product and the pressure medium increase in temperature, but the incompressible steel vessel does not. As a result, the product fraction near the vessel wall cools down and does not reach the same temperature as the product fraction in the centre of the vessel. This heat loss can compromise the effectiveness of the process by

allowing the sample temperature to drop below the level of lethality necessary to ensure product safety. Different technical solutions may be implemented to prevent this heat loss.

Table 1.1: Temperature changes of selected substances due to heating during physical compression (temperature rise per 100 MPa pressure increase) (De Heij *et al.*, 2003).

Substance	Initial temperature (°C)	Temperature change (°C/100 MPa)
Water	20	2.8
	60	3.8
	80	4.4
Steel	20	~0
Chicken	20	2.9
Cheese (Gouda)	20	3.4
Milk fat	20	8.5

System requirements for HP/HT conditions

A HPP system designed for commercial sterilization purposes must fulfil specific requirements (Barbosa-Canovas and Juliano, 2008). Pressure vessels must be able to withstand pressures within the range of 600 – 800 MPa. This can be achieved by using multilayer, wire-wound, or other pre-stressed designs. In these designs, vessels are deliberately fabricated with residual compressive stress to lower the maximum stress level in the vessel wall during pressurization (Mertens and Deplace, 1993). In addition, heat loss toward the vessel wall and pressure medium has to be avoided as heat contributes to the process's lethality. Successful installation of several features for heat loss prevention can make the system close to adiabatic and, in this way, maximize preservation efficacy at chosen HP/HT conditions (Hoogland *et al.*, 2001; De Heij *et al.*, 2003; Barbosa-Canovas and Juliano, 2008). Heat loss from the product to the colder pressure medium entering the vessel can be avoided by heating the HP pipes and/or the HP pump (in case of an external intensifier), by using an internal intensifier, or by avoiding thermal contact between the pressure medium and the product, e.g. by inserting the product into a specific container. Heat loss from the product to the pressure medium during pressure treatment can be prevented by choosing a pressure medium with an adiabatic temperature rise that is equal to or greater than that of the product. Thermal losses to the vessel wall can be limited by preheating the vessel at a temperature higher than the initial sample temperature (i.e. the temperature just before pressurization), by applying a high pressurization rate (> 5 MPa/s), or by thermal insulation of the internal vessel surface.

Bacterial spore inactivation

Thermal sterilization processes are designed to deliver at least a 12 log reduction of proteolytic *C. botulinum* spores. As heat resistance does not correlate with pressure resistance, it is not sure whether these pathogenic spores are also the most resistant in the T-p range of HP sterilization (Ganzle *et al.*, 2007). Moreover, pressure resistance varies strongly within strains of one species. Nevertheless, *C. botulinum* spores appear to be amongst the most pressure resistant (Margosch *et al.*, 2004). Further research is indispensable to determine the target organism in HP sterilization processes.

The effect of pressure during HP/HT processing may be characterized as being synergistic, not significantly different, or protective compared to thermal inactivation (Bull *et al.*, 2009). Most studies have concluded that pressure and heat do act synergistically to deliver lethality (Margosch *et al.*, 2006; Ahn *et al.*, 2007). Recently, Bull *et al.* (2009) found that pressure acts synergistically with heat in the inactivation of *C. botulinum* spores but the effect was strain and product dependent. Because synergy was not consistently observed among strains of *C. botulinum* or among products, the prediction of inactivation of *C. botulinum* spores by HP sterilization for the present must assume a complete lack of synergy. Hence, based on the current knowledge, regulatory approval can only be obtained by filing this technology as a thermal process (Barbosa-Canovas and Juliano, 2008). Therefore, any HP sterilization process for shelf stable low-acid foods must be at least thermally equivalent to a process value F_0 of three minutes. A standard scenario for commercial HP sterilization could be the combination of 700 MPa and a final process temperature of 121.1 °C with a holding time of three minutes (Sizer *et al.*, 2002; Barbosa-Canovas and Juliano, 2008). In this case, the required lethality is obtained by only accounting for the thermal component and neglecting the pressure effect on microbial inactivation. To gain regulatory approval for a HP sterilization process that takes into account a lethal effect of pressure more microbial inactivation data on many *C. botulinum* strains as well as surrogate spore-forming micro-organisms are needed (Patazca *et al.*, 2006; Lau and Turek, 2007; Ganzle *et al.*, 2007).

HP/HT processing and food quality

As stated above, it has not yet been univocally demonstrated whether the elevated pressure in HP sterilization acts synergistically with the high-temperature for spore inactivation or whether the benefit of elevated pressure is solely the rapid, uniform heating during pressurization and, correspondingly, cooling during depressurization (Barbosa-Canovas and Juliano, 2008). The former will result in lower maximum temperatures and shorter treatment times. In the latter case, treatment times will be shorter but maximum temperatures will be retained. Either way, a better retention of quality characteristics susceptible to heat is expected. However, the variable pressure by itself might influence particular

qualities. For example, pressure may accelerate or decelerate (depending on the negative or positive reaction volume) reactions taking place at the applied process temperature.

Even though extensive research has been done on bacterial spore inactivation, quality validation studies of low-acid foods after HP/HT treatment are rather limited. Moreover, the processing conditions studied have not been proven to really result in food safety and shelf stability. Studies have demonstrated for example better retention of flavour components in fresh basil (Krebbers *et al.*, 2002a), firmness in green beans (Krebbers *et al.*, 2002b; Leadley *et al.*, 2008), and colour in tomato puree (Krebbers *et al.*, 2003) after HP/HT processing. However, these studies used a two-pulsed pressure treatment (Meyer, 2000) which, from an economical perspective, is not recommended. More research on the effect of HP/HT processing on nutritional and sensory food quality is required. These studies are likely to be needed on a product by product basis (Matser *et al.*, 2004) and clearly need to be constrained within processing conditions known to yield commercial sterility and of industrial relevance (Leadley *et al.*, 2008).

Analogously to HP pasteurization where direct product scaling-up to larger volumes of food is possible due to the uniform and instantaneous pressure transmission, HP/HT processing is suitable for larger sizes as compression heating to high-temperatures is instantly achieved throughout the entire package volume. However, a uniform temperature increase during compression can only be achieved in case of a uniform initial product temperature. An extended preheating time, especially in a large container, to obtain a uniform initial temperature, may affect the quality characteristics of the food product. So in order to maximize quality retention, it is desirable to minimize the duration of preheating needed to reach the target initial temperature (Barbosa-Canovas and Juliano, 2008). By applying higher pressure levels, the required initial product temperature can be lowered, hereby enhancing food quality (Lau and Turek, 2007).

1.2 TEXTURE OF FRUITS AND VEGETABLES

Texture is one of the most important quality characteristics of fruit and vegetable based products. Although the term is widely used, texture is not a single, well-defined attribute (Szczesniak, 2002). It is a collective term that encompasses the structural and mechanical properties of a food and their sensory perception in the hand or mouth (Abbott and Harker, 2002). People sense texture in numerous ways: the look of the product, the feel in the hand, the way it feels as they cut it, the sounds as they bite and chew, and most important of all, the feel in their

mouth as they eat it (Abbott and Harker, 2002). A few of the many terms used to describe sensory texture of fruits and vegetables are hard, firm, soft, crisp, mealy, though, dry, and juicy. Sensory texture assessment involves the codification of sensory experiences, and is commonly carried out by trained panels. The desire to monitor and evaluate texture during food production and processing has led to the development of instrumental measurement methods (Waldron *et al.*, 2003). Instruments are more convenient to use, more precise, less expensive, and widely available, tend to provide consistent values when used by different (often untrained) people, and can provide a common language among researchers, industry, and customers. It is often suggested that the relevance of instrumental measurements depends on how well they predict sensory attributes. However, they can also be valuable for mechanical property measurements that relate only to the functional behaviour of the fruit or vegetable (e.g. bruise resistance) (Abbott and Harker, 2002).
The underlying plant structure is a key determinant of plant texture (Waldron, 2004). Edible fruits and vegetables are generally rich in weak, non-specialized parenchyma cells. The texture of such tissues is mainly determined by the structural integrity of the primary cell wall and middle lamella, as well as by the turgor pressure generated within cells by osmosis (Van Buren, 1979; Jackman and Stanley, 1995; Brett and Waldron, 1996; Waldron, 2004). Both factors are affected by processing such as heating, which can result in tissue over-softening. Loss of structural integrity is mainly due to depolymerisation of cell wall pectic polysaccharides resulting in a weakened cell adhesion (Waldron *et al.*, 1997b). Consumers' demand for 'fresh-like' processed fruits and vegetables has stimulated research on ways of controlling the textural quality of processed fruits and vegetables.
This section deals with the texture of fruits and vegetables and how this quality parameter is influenced by processing. First, plant structure and more specifically the plant cell wall are described. Subsequently, textural changes during thermal and HPP are discussed in relation to the underlying pectin conversions. Finally, techniques applied for texture improvement of thermally processed fruits and vegetables are outlined.

1.2.1 PLANT STRUCTURE

1.2.1.1 A structural hierarchy

Fruit and vegetable texture is derived from a structural hierarchy (Figure 1.2) (Jackman and Stanley, 1995; Waldron *et al.*, 1997b; Waldron *et al.*, 2003). The lowest level of structure to be considered is that of the polymers that make up the plant cell wall. How they are arranged (e.g. as matrix polysaccharides or complex

cellulose microfibrils) and interact, both physically and chemically, largely determines the mechanical properties of the wall. The latter is also influenced by cell wall thickness and by structures within it, for example plasmodesmata and patterns of secondary thickening. Cells constitute the next level of structure. These differ in shape and size depending on their function. Cells are joined to one another at the middle lamella; the extent and strength of adhesion can vary. The rigidity of individual cells is affected by the elasticity of the wall which, in conjunction with the osmotic potential of the cell contents and availability of water, determines turgor pressure. Cells and intercellular spaces (air or water filled) are arranged into tissues, whose shape and arrangement comprise the next level of structure. The textural characteristics of the final organ depend on contributions from the different levels of structure, and how they interact with one another.

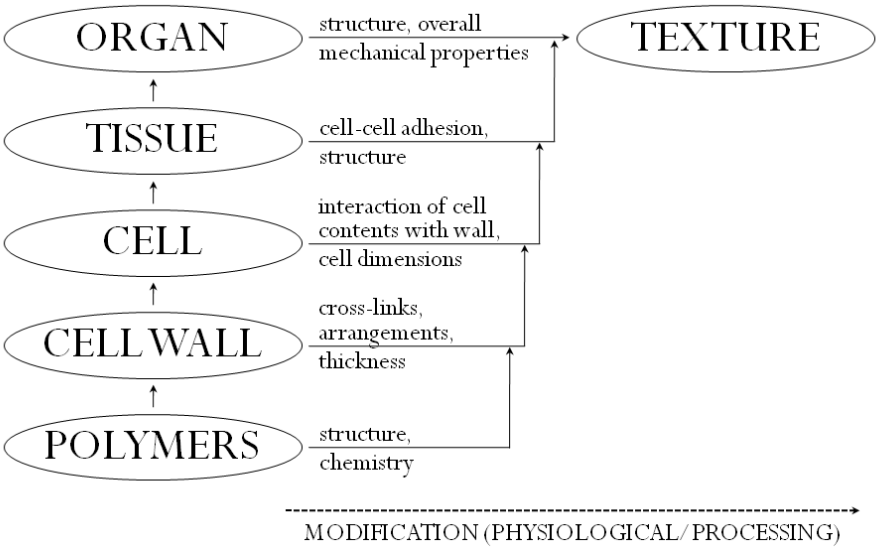


Figure 1.2: Schematic representation of the levels of structure that contribute to the mechanical properties of plant tissues (Waldron *et al.*, 1997b).

1.2.1.2 Cell wall composition

The plant cell wall consists of several layers (Brett and Waldron, 1996). The earliest-formed, outer layer found at the centre of the double wall formed by two adjacent cells is the middle lamella. During cell growth, both cells deposit the next major layer, the primary cell wall. Certain specialized cells lay down a third layer at the onset of differentiation, the secondary cell wall. The latter is virtually

absent from edible plant tissues (Van Buren, 1979). Figure 1.3 shows a simplified and schematic representation of the cell wall of fruits and vegetables.

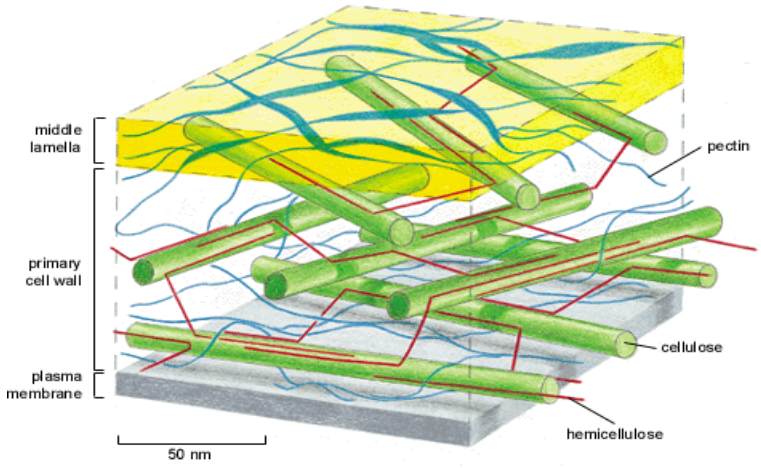


Figure 1.3: A simplified, schematic representation of the cell wall of fruits and vegetables (McCann and Roberts, 1996).

Each layer consists of a microfibrillar phase and a matrix phase (Fry, 1986; Brett and Waldron, 1996). The microfibrils are made up of about 30 to 100 cellulose molecules, which are aligned parallel to the long axis of the microfibril. Cellulose is an unbranched β-(1→4) glucan with a degree of polymerisation of between 2000 and 6000 in primary cell walls to more than 10000 in secondary walls (Waldron *et al.*, 2003). The glucan chains interact closely through hydrogen bonding, excluding water to produce areas of crystallinity. These impart considerable tensile strength (Brett and Waldron, 1996). The matrix phase largely consists of pectin, hemicelluloses, proteins, phenolics, and water (Van Buren, 1979; Brett and Waldron, 1996).

Pectin is a group of polysaccharides rich in galacturonic acid (GalA), rhamnose (Rha), arabinose (Ara) and galactose (Gal) (Brett and Waldron, 1996), although they can be composed of as many as 17 different monosaccharides (Vincken *et al.*, 2003). These monosaccharides are organized in a number of distinct polysaccharides. The three major pectic polysaccharides are homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). HG is a linear chain of (up to 200) 1,4-linked α-D-GalA residues, 70 to 80% of them are methyl esterified at the C-6 carboxyl group on insertion into the cell wall (Willats *et al.*, 2001; Waldron *et al.*, 2003). The degree of methoxylation (DM) is a major factor determining pectin functionality (Willats *et al.*, 2006). Depending on the plant source, HG can also be O-acetylated on C-2 and/or C-3 (Willats *et*

al., 2001; Ridley *et al.*, 2001). RG-I comprises up to 100 repeats of the disaccharide [→4)-α-D-GalA-(1→2)-α-L-Rha-(1→] (Willats *et al.*, 2001). It is not clear whether GalA residues in RG-I can be methoxylated (Willats *et al.*, 2001; Ridley *et al.*, 2001). A large proportion (20 – 80%) of the Rha residues are substituted at C-4 with side chains, principally composed of Ara and Gal. These side chains may be branched and complex, and RG-I domains are often described as "hairy" regions when compared with the "smooth" regions of HG (Waldron *et al.*, 2003). RG-II has a HG backbone that is substituted by four heteropolymeric side chains of known and consistent composition (Willats *et al.*, 2001; Ridley *et al.*, 2001; Vincken *et al.*, 2003). Besides these three main polysaccharides, other substituted galacturonans, such as xylogalacturonans and apiogalacturonans, have been described that are present in the walls of a restricted number of plants (Willats *et al.*, 2001; Ridley *et al.*, 2001; Vincken *et al.*, 2003). Although the fine structure of the various pectic constituents is largely known, it is still unclear how these structural elements are combined into a macromolecular structure. The constituent polymers are covalently linked to each other (Coenen *et al.*, 2007; Mohnen, 2008). For a long time, it was accepted that HG, RG-I and RG-II are linearly connected to form a continuous backbone. However, in 2003, Vincken *et al.* (2003) postulated an alternative macromolecular structure in which HG is a side chain of RG-I. No conclusive evidence confirming or excluding either of the structures has been presented, although the currently available information strongly supports the first model (Coenen *et al.*, 2007; Mohnen, 2008). Additional research is needed to elucidate the macromolecular build-up of this polymer.

Hemicelluloses are defined as non-cellulosic cell wall polysaccharides other than pectins which can be extracted by alkaline solutions, needed due to strong hydrogen bonding between the hemicellulose and cellulose microfibrils (Brett and Waldron, 1996). Their composition can differ greatly in different cell types and in different species (Brett and Waldron, 1996). Xyloglucan is the prominent hemicellulose in the cell walls of dicotyledonous plants. Monocotyledonous plants contain arabinoxylan as a major hemicellulose (Waldron *et al.*, 2003).

Wall proteins can be classified into two groups: enzymes and structural proteins (Waldron *et al.*, 2003). The enzymes have a range of functions, including polymer turnover, wall degradation, and wall remodelling associated with plant growth, development, maturation, and senescence (Waldron *et al.*, 2003). Most structural proteins are glycosylated and the most abundant ones contain the unusual amino acid hydroxyproline (Brett and Waldron, 1996). These hydroxyproline-rich glycoproteins (HRGPs) comprise several groups; the most extensively studied group is known as extensin. HRGPs contain tyrosine residues which are able to cross-link in the wall to form intra- and intermolecular covalent bridges (Fry, 1986).

Cell wall phenolics can be divided broadly into two main classes. The major class, known as lignin, is produced from oxidative cross-linking of phenolic alcohols. The result is a very strong, hydrophobic mesh work which surrounds the other wall components and cements them in place. The second class consists of relatively simple phenolic acids (e.g. ferulic acid) which are attached to wall polysaccharides, and which can be used for producing covalent cross-links through peroxidative and related activity (Brett and Waldron, 1996; Waldron *et al.*, 2003).

Water is also a large component of cell walls. Water is believed to have four major functions in the wall (Van Buren, 1979). (i) It is a structural component as part of the matrix gel. (ii) It can serve as a wetting agent, interrupting direct hydrogen bonding between polymers. (iii) It can cooperate in stabilising conformations of polymers. (iv) It serves as a solvent or transport medium for salts, low-molecular-weight compounds and enzymes.

The relative composition of the wall varies from the cell membrane to the middle lamella (Van Buren, 1979). In the primary wall of fruits and vegetables there are approximately equal amounts (one-third of the dry weight each) of pectin, hemicelluloses and cellulose. The cellulose has the function of giving rigidity and resistance to tearing, while the pectin and hemicelluloses confer plasticity and the ability to stretch. The middle lamella may be considered an extension of the matrix material of the primary cell wall, with in particular pectin abundantly present. As the outermost portion of the plant cell, it plays the primary role in intercellular adhesion.

1.2.1.3 Cell wall architecture

A recent model of cell wall architecture visualises the cell wall as containing a number of structurally independent polymer networks which, when superimposed upon one another, interact further to give rise to the whole, complex cell wall structure (Carpita and Gibeaut, 1993; Jackman and Stanley, 1995; Brett and Waldron, 1996; McCann and Roberts, 1996; Kunzek *et al.*, 1999). Although the fine structure of the individual cell wall components has been identified, exact knowledge on the interactions of these components is rather limited (Fry, 1986; Sila *et al.*, 2009). It seems that hydrogen bonds, Ca^{2+} bridges, other ionic bonds, coupled phenols, and ester bonds all play a role in building the wall, although other cross-links may also be involved (Fry, 1986). In non-lignified plant cell walls three networks are present: the cellulose-hemicellulose, the pectin and the extensin network.

The cellulose-hemicellulose network is made up of hemicelluloses that cross-link cellulose microfibrils through hydrogen bonding (Brett and Waldron, 1996). This network provides the main structural strength in growing cell walls.

The pectin network is formed by various covalent and non-covalent cross-links (Brett and Waldron, 1996; Vincken *et al.*, 2003; Mohnen, 2008; Sila *et al.*, 2009). Two unesterified HG chains can engage in a complex, in which the carboxyl groups of two GalA residues form a negatively charged pocket that can accommodate a divalent cation, particularly Ca^{2+}. At least nine contiguous unesterified GalA residues are required to build a stable cross-link between chains. Two molecules of RG-II can complex with boron, forming a borate-diol ester. HG can be cross-linked to other components by uronyl esters. Particular pectin methylesterases can catalyse a transesterification reaction, using methyl esterified HG as a donor substrate. In *Chenopodiaceae*, pectin can be cross-linked through esterification with ferulic acid. Ferulic acid is esterified to Ara and Gal in RG-I, and two ferulic acid units can be linked by peroxidase activity to form a diphenyl bond. Hydrophobic interactions between methoxyl groups and hydrogen bonds between undissociated carboxyl and secondary alcohol groups may also occur in the cell wall.
The HRGP extensin forms a third network in the cell wall. The nature of the cross-links is not known for certain but probably involves tyrosine residues. Extensins are oriented perpendicular to the plasma membrane and hence to the cellulose microfibrils. They may thus hold the different layers together (Brett and Waldron, 1996).
Since nearly all cell wall macromolecules are large and many tend to take up an irregular, extended conformation, it is likely that spatial entanglement will help to prevent relative movement between them and hence contribute to the strength of the wall (Fry, 1986; Brett and Waldron, 1996).

1.2.2 TEXTURAL CHANGES DURING PROCESSING

1.2.2.1 The role of pectin conversions

Generally, in view of textural changes, pectin is regarded to be the most important cell wall polymer because of its abundance, its solubility and its sensitivity to chemical reactions (Van Buren, 1979; Sila *et al.*, 2008). As pectin is particularly abundant in the middle lamella, it plays a crucial role in cementing the cells together.
Pectin can undergo a variety of chemical conversions under conditions similar to those associated with food processing (Van Buren, 1979). These modifications include demethoxylation and depolymerisation reactions, and can be respectively beneficial or detrimental for the texture of fruits and vegetables. During processing, pectin is also susceptible to biochemical changes. By boosting the activity of certain functionally important pectinases, texture can be improved

(Van Buggenhout *et al.*, 2009) (see section 1.2.2.4). In this context, PME and its processing stability and activity are of major interest (Duvetter *et al.*, 2009).

Chemical demethoxylation

Demethoxylation of the esterified carboxyl groups in HG mainly proceeds through saponification under mild acid or alkaline conditions (Van Buren, 1979). Since saponification is initiated by hydroxyl ions, the reaction rate increases with increasing pH (Van Buren, 1979; Kravtchenko *et al.*, 1992; Renard and Thibault, 1996). The rate of saponification is proportional to the amount of esters remaining, in other words, the reaction rate declines as the reaction proceeds (Renard and Thibault, 1996; Fraeye *et al.*, 2007a). The latter is also partly the result of an increasing repulsion of hydroxyl ions (by the liberated carboxyl groups) (Albersheim *et al.*, 1960). The rate of demethoxylation increases with increasing temperature (Van Buren, 1979; Kravtchenko *et al.*, 1992; Diaz *et al.*, 2007; Fraeye *et al.*, 2007a). Verlent *et al.* (2004) observed a stimulating effect of HP on the demethoxylation at pH 8.0 for pressures and temperatures up till 500 MPa and 65 °C. This enhancing effect can be explained by the principle of Le Chatelier. Solvation of the charged groups created by the pectin demethoxylation is accompanied by a reduction in reaction volume resulting from electrostriction (i.e. the compact alignment of water dipoles owing to the coulombic field of the charged groups). Chemical demethoxylation results in a random distribution of free and methoxylated GalA residues (Van Buren, 1979).
In addition, demethoxylation can occur under acid conditions through a mechanism of acid hydrolysis. However, few studies have focused on this (Fraeye *et al.*, 2007a).

Acid hydrolysis

Pectin with a low DM can be subject to acid hydrolysis during thermal treatment. This reaction proceeds through an initial protonation of the glycosidic oxygen to give the conjugated acid. This is followed by a rate-limiting unimolecular heterolysis of the conjugated acid with the formation of a non-reducing end group and a carbenium ion. Rapid addition of water to the carbenium ion results in the formation of a reducing end group and a proton (Smidsrod *et al.*, 1966; Van Buren, 1979). Krall and McFeeters (1998) and Fraeye *et al.* (2007a) observed rather fast hydrolysis at pH 2.0 and declining reaction rates with increasing pH. The rate of acid hydrolysis increases with increasing temperature (Diaz *et al.*, 2007; Fraeye *et al.*, 2007a) and decreasing pectin DM (Krall and McFeeters, 1998; Diaz *et al.*, 2007; Fraeye *et al.*, 2007a). Kato *et al.* (1997) reported that no acid hydrolysis takes place during HPP (700 MPa) at room temperature at pH 1.0 or higher.

Beta-eliminative depolymerisation

Splitting of glycosidic linkages between GalA residues takes place by a process of beta-elimination during heating at neutral or alkaline pH (Van Buren, 1979). This reaction proceeds on uronic acids, which possess a glycosidic linkage on C-4 in the β-position of the carboxyl group at C-5 (Albersheim *et al.*, 1960; BeMiller and Kumari, 1972; Kiss, 1974). A prerequisite is the presence of a methyl ester group at C-6, rendering H-5 sufficiently acidic to be removed by a hydroxyl ion. This results in the formation of unstable, intermediary anions that are stabilized by losing the C-O linkage in the β-position. Consequently, a double bond appears between C-4 and C-5 at the non-reducing end (Figure 1.4).

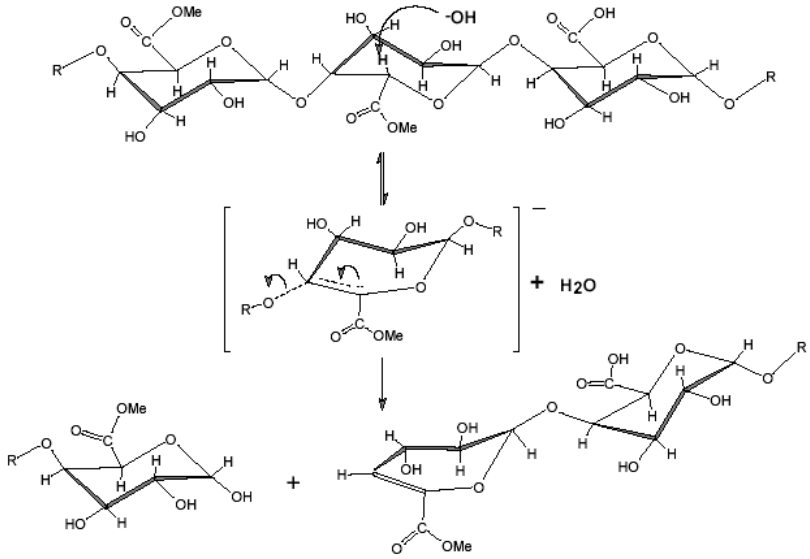


Figure 1.4: The beta-elimination reaction mechanism on pectin (adapted from Keijbets and Pilnik, 1974).

Pectin with a high DM is more susceptible to beta-elimination than pectin with a low DM (Albersheim *et al.*, 1960; Sajjaanantakul *et al.*, 1989; Krall and McFeeters, 1998; Fraeye *et al.*, 2007a). It is important to note that, during heating of pectin, simultaneous chemical demethoxylation can occur. Hence, the DM of the pectin (and the pH) decreases, causing a decrease in the beta-elimination rate (Kravtchenko *et al.*, 1992; Fraeye *et al.*, 2007a).

Beta-elimination has been measured at pH values as low as 3.5 (Krall and McFeeters, 1998; Sila *et al.*, 2006b). Reaction rates increase with increasing pH because hydroxyl ions initiate the reaction (Kravtchenko *et al.*, 1992; Fraeye *et al.*, 2007a). Cations also promote the beta-elimination reaction (Keijbets and

Pilnik, 1974; Sajjaanantakul *et al.*, 1993). The extent of cleavage increases roughly with ion concentration and valency. The effect is more pronounced for low-methoxyl pectin than for high-methoxyl pectin. It has been suggested that ions may associate with free carboxyl groups on the pectin, resulting in a decrease in the overall, negative charge, which may facilitate the approach of hydroxyl ions needed to initiate beta-elimination (Keijbets and Pilnik, 1974; Sajjaanantakul *et al.*, 1993). Beta-elimination is also stimulated by organic anions, e.g. citrate, malate, phytate, although no explanation for this observation has been formulated yet (Keijbets and Pilnik, 1974).
The rate of beta-elimination increases with temperature. Activation energies ranging from 80 to 136 kJ/mol have been reported (Sila *et al.*, 2006b; Diaz *et al.*, 2007; Fraeye *et al.*, 2007a). These rather high values indicate that the reaction rate constant strongly depends on temperature. During HPP (700 MPa) at room temperature, no beta-elimination takes place at pH values up to 13.0 (Kato *et al.*, 1997).

Pectin methylesterase

PME is frequently employed as a biotechnological tool for reducing thermal texture loss in fruits and vegetables (Duvetter *et al.*, 2009; Van Buggenhout *et al.*, 2009) (see section 1.2.2.4). PME catalyses the hydrolysis of methyl esters at C-6 of GalA residues in HG. It is produced by most higher plants, as well as by many phytopathogenic micro-organisms. It has been described that PMEs with alkaline pI (mostly plant PME) demethoxylate "blockwise", generating long sequences of non-methoxylated GalA residues, whereas PMEs with acidic pI (mostly microbial PME) demethoxylate more randomly (Markovic and Kohn, 1984). Each PME type, depending on its biological origin and/or isozyme is characterized by a certain p-T stability window (Duvetter *et al.*, 2009). Generally, PMEs are rather thermo-labile since most of the (purified) enzymes readily inactivate at temperatures below 70 °C. Contrary to their temperature sensitivity, most PMEs are barotolerant. Nonetheless, the pressure stability of PME can vary considerably ranging from the moderately pressure-sensitive types (> 600 MPa) like carrot PME (Ly-Nguyen *et al.*, 2003; Sila *et al.*, 2007a; Jolie *et al.*, 2009) to the extremely barotolerant ones (> 1 GPa) like tomato PME (Crelier *et al.*, 2001). As opposed to the purified form, PME in tissue-type systems is less vulnerable to thermal or HP inactivation (Balogh *et al.*, 2004; Sila *et al.*, 2007a). Pressure and temperature often exhibit an antagonistic effect on PME stability; moderate pressure retards the thermal inactivation (Ly-Nguyen *et al.*, 2003). Pectin demethoxylation by PME is enhanced by increasing temperature until a point is reached where enzyme inactivation interferes. Optimal temperatures for PME catalytic activity vary between 45 and 55 °C depending on the enzyme source and environment (Duvetter *et al.*, 2009). A 2- to 6-fold increase in carrot PME catalytic activity can be achieved by increasing the temperature from 20 to

55 °C (Sila *et al.*, 2007a). For all PMEs, the substrate conversion rate increases within a certain p-T combination window (Sila *et al.*, 2008; Duvetter *et al.*, 2009). In the case of carrot PME, the optimal conditions for substrate conversions were 50 °C in combination with 300 to 500 MPa in model systems; however, in tissue-type systems the optimal temperature shifted to 60 °C in combination with 200 to 400 MPa (Sila *et al.*, 2007a). The stimulating effect of pressure on PME activity can be explained by the principle of Le Chatelier.

1.2.2.2 Thermal processing of fruits and vegetables

Thermal processing of fruits and vegetables results in considerable softening. During the process, several changes occur and these can impact on one or more parts of the structural hierarchy (Waldron *et al.*, 2003). An initial loss of instrumental firmness is due to membrane disruption and the associated loss of turgor (Greve *et al.*, 1994b). This may result in the development of a rubbery character. However, the most significant softening occurs subsequently as a result of an increase in the ease of cell separation (Ng and Waldron, 1997a). Thermally induced cell separation is believed to be due to beta-eliminative degradation of the pectic polysaccharides in the middle lamella (Van Buren, 1979; Greve *et al.*, 1994a; Waldron *et al.*, 2003). This depolymerisation leads to pectin solubilisation, resulting in loss of structural integrity. Since the pH of plant cell walls is generally between 4.0 and 6.0 (Brett and Waldron, 1996), it can be stated that during thermal treatment of plant-based foods the occurrence of acid hydrolysis of pectin is negligible compared to the beta-elimination reaction (Van Buggenhout *et al.*, 2009). Sila *et al.* (2006b) and Vu *et al.* (2006) found a very strong positive correlation between the thermal texture degradation rate constant and the rate constant of the beta-eliminative reaction indicating that beta-elimination is the main contributing factor to thermal texture degradation. Linked to the beta-eliminative depolymerisation of pectin, changes in pectin solubility occur during thermal processing (Sila *et al.*, 2009). These changes can be assessed using sequential extractions with different solvents, such as water, aqueous solutions of chelating agents, and aqueous solutions of alkali (Selvendran and Oneill, 1987). HT processing is accompanied by an increase in water soluble pectin and a concomitant decrease in cyclohexane-trans-1,2-diamine tetra-acetic acid (CDTA) soluble, Na_2CO_3 soluble, and residual pectic polysaccharides (Greve *et al.*, 1994a; Siliha *et al.*, 1996; Stolle-Smits *et al.*, 1997; Ng and Waldron, 1997a; Ng and Waldron, 1997b; Sila *et al.*, 2006a). Moreover, small quantities of pectin are released into the cooking liquor. In addition to the extractability of the pectins, the composition of the different fractions is modified (Stolle-Smits *et al.*, 1997; Ng and Waldron, 1997a). A thermal process results in an increase in the GalA to neutral sugars ratio of water soluble pectin and a concomitant decrease in this ratio in the subsequent extracts, indicating that the heat treatment is

solubilising GalA rich polysaccharide moieties. It is likely that the more highly branched pectic polymers are more highly cross-linked, and are more readily retained by the cell wall. At the microscopic level, thermal processing of plant tissues results in cell wall swelling. Likewise, a clear transition from cell rupture to cell separation can be seen with increasing thermal impact (Stolle-Smits *et al.*, 1998; Waldron *et al.*, 2003; Sila *et al.*, 2007b).

Thermally induced cell separation occurs predominantly in non-lignified, thin-walled tissues. Tissues that fail to soften often consist of cells that have undergone secondary thickening and associated lignification. However, some edible, non-lignified parenchyma tissues do not soften or soften extremely slowly during thermal processing. These include Chinese water chestnut (CWC) and, to a lesser extent, sugar beet and beetroot (Waldron *et al.*, 1997a; Waldron *et al.*, 1997b). After thermal treatments, they largely retain their firm and crisp texture in terms of both sensory perception and mechanically-measured tensile strength and toughness. This is because its cells fail to separate and tissue failure occurs only by cell wall fracture. It appears that in CWC arabinoxylans cross-linked by ferulic acid are responsible for thermal stability of texture. Sugar beet and beetroot, both dicotyledonous plants, exhibit similar characteristics as a result of the ferulate cross-linking of pectic polysaccharides. This cross-linking is the result from the action of cell wall-bound peroxidase in the presence of H_2O_2.

1.2.2.3 High-pressure processing of fruits and vegetables

The effect of HPP on texture of fruits and vegetables is largely dependent on product type and pressure level. In general, textural changes occur in two phases (Basak and Ramaswamy, 1998). There is an instantaneous initial loss of firmness as a result of the pulse action of pressure, with higher pressures resulting in greater losses. At low pressures (100 MPa), instantaneous pressure softening is caused by compression of cellular structures without disruption, while at higher pressures (> 200 MPa) severe texture loss occurs owing to rupture of cellular membranes and consequent loss of turgor pressure. During pressure holding, further loss or gradual recovery can occur. Gain in firmness has been attributed to increased compactness of the cellular structure following degassing of the tissue as well as to PME activity resulting in increased pectin cross-linking and consequently enhanced cell adhesion (Basak and Ramaswamy, 1998). Generally, the effect is highly dependent on the plant type, and its morphological and structural features (Van Buggenhout *et al.*, 2009). For instance, plants with a firm intact structure such as cauliflower reveal nearly fresh texture after HP treatment at 400 MPa in combination with 5 °C (Prestamo and Arroyo, 1998), whereas highly porous fruits such as strawberries show structure collapse when treated at 400 MPa in combination with 10 °C (Duvetter *et al.*, 2005).

Kato *et al.* (1997) compared the effects of HPP (100 – 700 MPa, 20 °C, 45 min) and cooking on texture and pectin composition of carrots. When comparing raw carrots with pressurized carrots, firmness was the same but pressurization (especially from 200 MPa on) increased rupture strain, indicating a more rubbery character of pressurized carrots. Similar observations were made by Araya *et al.* (2007). After 30 min cooking, total carrot pectin (in particular highly methoxylated pectin) decreased, due to pectin depolymerisation and solubilisation. Total pectin in pressurized carrots was found to be the same as that in 3 min cooked carrots, indicating limited solubilisation. With increasing pressure, high-methoxyl pectin in carrots decreased while low-methoxyl pectin increased, suggesting PME activity occurred. HP processed carrot tissue showed no cell wall swelling. However, cells were more irregular in shape than raw carrot tissue, probably due to compression and decompression effects during processing (Araya *et al.*, 2007).

Few studies have investigated the effect of combined HP/HT processing on the texture of fruits and vegetables. Nguyen *et al.* (2007) compared the effectiveness of HP/HT processing with that of conventional thermal processing in preserving carrots' texture. Both HT and HP/HT treated samples exhibited initial tissue softening during process come-up time followed by further softening during subsequent holding time. HT treated samples lost more hardness than HP/HT samples for the different process temperatures (95, 105, 121 °C) studied. With increasing temperature and pressure, carrot tissue softening increased. However, at 121 °C texture loss was more pronounced at 500 MPa than at 700 MPa, illustrating the importance of the initial product temperature (i.e. the temperature just before pressurization). If this temperature is relatively high (depending on process temperature and pressure), thermal effects can predominate.

1.2.2.4 Texture improvement of thermally processed fruits and vegetables

Consumers' demand for 'fresh-like' processed fruits and vegetables has prompted progressive research on ways of improving the texture of thermally processed fruits and vegetables. In this context, maintenance of cell adhesion is of crucial importance (Waldron *et al.*, 2003). This can be achieved in two ways: by minimizing pectin degradation during processing and/or by strengthening the middle lamella matrix prior to processing (Van Buggenhout *et al.*, 2009).

Minimization of beta-elimination

Since the beta-elimination reaction is highly dependent on the methyl ester content of pectin, targeted manipulation of the pectin DM can lead to controlled beta-elimination (Sila *et al.*, 2008). This can be achieved through genetic

engineering, more specifically by manipulation of pectin biosynthesis, or by a controlled activation of the enzyme PME using pretreatments (Van Buggenhout *et al.*, 2009).

Preheating at mild temperatures (50 – 70 °C) typically for 30 minutes or more reduces the vulnerability of the plant tissue to softening during subsequent HT processing (Van Buren, 1979; Smout *et al.*, 2005). Thermal stimulation of wall-bound PME demethoxylates pectic polysaccharides involved in cell adhesion. The reduction in methyl ester groups reduces the rate and extent of beta-eliminative degradation at high temperatures. The preheating effect can be sizeable in vegetables such as carrots (Ng and Waldron, 1997a; Smout *et al.*, 2005). In some others, e.g. potatoes, it is often less and it is often variety dependent. After preheating, the amount of water soluble pectin is decreased while the amount of CDTA and Na_2CO_3 soluble pectin is increased (Siliha *et al.*, 1996; Stolle-Smits *et al.*, 1997; Stolle-Smits *et al.*, 2000; Sila *et al.*, 2006a; Sila *et al.*, 2006b). During subsequent heating, heat-induced modifications (i.e. increase in water soluble pectin and decrease in CDTA and Na_2CO_3 soluble pectin) are reduced, revealing less pectin degradation (Ng and Waldron, 1997a; Ng and Waldron, 1997b; Stolle-Smits *et al.*, 2000).

Next to using HPP as a preservation unit operation, it can be applied as a preprocessing tool. A HP pretreatment at mild temperatures has proven to retard thermosoftening of carrots remarkably (Sila *et al.*, 2004; Sila *et al.*, 2005). The main reason for the increased positive effect of preheating when combined with pressures up to 400 MPa is increased catalytic activity of PME (Verlent *et al.*, 2004; Duvetter *et al.*, 2006; Sila *et al.*, 2007a) and, consequently, the extensive modification of pectins in terms of their DM and solubility (Sila *et al.*, 2006a).

The pretreatment strategy of stimulating endogenous PME activity is not effective when other detrimental enzymes present are also stimulated by the pretreatment or when only low amounts of endogenous PME are present (Van Buggenhout *et al.*, 2009). In these cases, the infusion of exogenous PME can be beneficial, especially in porous tissues like for example strawberries (Duvetter *et al.*, 2005).

Enhancement of cell adhesion

Another approach in the texture improvement of heat-treated fruits and vegetables is strengthening of the middle lamella matrix, which cements the parenchyma cells together (Van Buggenhout *et al.*, 2009). This can be achieved by soaking the products in a solution containing Ca^{2+}. Salt-bridge formation between Ca^{2+} and free carboxyl groups of pectin enhances cell adhesion. A reduction in pectin methyl ester groups provides greater opportunity for the pectic polymers to be ionically cross-linked by divalent ions such as Ca^{2+}, hence the frequent combination of a thermal or HP pretreatment and Ca^{2+} soaking (Smout *et al.*, 2005). Ca^{2+} soaking is most valuable in texture improvement when carried out after the thermal or HP pretreatment. The pretreatment damages the cell wall

structure, thereby making the cells more permeable (Sila *et al.*, 2005). Ca^{2+} has two opposite effects on texture. On the one hand, it firms the tissue through cross-linking of pectic substances. On the other hand, it increases tissue softening by enhancing the beta-elimination reaction. The net result of Ca^{2+} addition, however, has been to firm the tissue (Van Buren, 1979).
Increased cross-linking of pectin chains by means of ferulic acid bridges might be another way to enhance cell adhesion (Waldron *et al.*, 1997b). Incubation of beetroot tissue in a H_2O_2 solution prior to cooking resulted in retarded thermosoftening due to enhanced phenolic acid cross-linking (Ng *et al.*, 1998). However, most fruits and vegetables contain only minor levels of ferulic acid. Therefore, an increase in phenolic acid cross-linking will require an increase in ferulic acid attached to polysaccharides at appropriate locations in the cell wall. In addition, enzymes that are able to cross-link ferulic acid and those involved in the production of H_2O_2 have to be present (Waldron *et al.*, 1997b).

1.3 CONCLUSION AND SCOPE OF THIS WORK

In the quest for food products with an extended shelf life but with similar quality characteristics as the "fresh", unprocessed product, HPP has proven beneficial. Currently, this technology is finding its way to the consumer as indicated by the increasing number of commercial applications and industrial HPP machines. At present, HPP is restricted to chilled or mild temperatures and the main food application is pasteurization. The application window may be extended to sterilization by combining HP with elevated temperature. Although extensive research has been done on bacterial spore inactivation, quality validation studies regarding HP/HT processing are rather limited. As pressure introduces an additional processing variable (compared to thermal processing at 0.1 MPa), quality characteristics might be affected differently. In this work, the effect of HP/HT processing on the quality parameter texture is studied as the latter is a major quality attribute of fruit and vegetable based products and is significantly changed by a thermal process. By studying thermal processing at 0.1 MPa and HP/HT processing, this work aims at understanding (mechanisms) and quantifying (kinetics) the effect of elevated pressure on texture of fruit and vegetable based products.

2 MECHANISTIC INSIGHT INTO TEXTURE EVOLUTION OF HIGH-PRESSURE/HIGH-TEMPERATURE PROCESSED CARROTS[1]

2.1 INTRODUCTION

Thermal processing of fruits and vegetables can (depending on the process intensity required to reach microbial safety) result in deleterious nutritional and sensory quality changes (e.g. tissue over-softening). HPP has emerged as a strong alternative assuring safety while minimizing quality losses. In the context of texture preservation of processed fruits and vegetables, HPP is valuable both as preprocessing technology and actual preservation (i.e. pasteurization) unit operation (see chapter 1).

Sila *et al.* (2006a; 2006b) carried out a detailed mechanistic study (including macroscopic, microscopic and molecular observations) on texture evolution of thermally processed carrots, including the effect of a HP pretreatment (60 °C/400 MPa). This chapter can be seen as a continuation of their work. The effect of HPP in combination with slightly elevated temperature (HP/HT processing) representing the actual preservation process (rather than preprocessing tool) was investigated with an eye to HP sterilization. A detailed picture was obtained by abstracting information at macroscopic, microscopic and molecular levels.

[1] This chapter is based on the following paper:
De Roeck, A., Sila, D.N., Duvetter, T., Van Loey, A., Hendrickx, M. (2008). Effect of high pressure/high temperature processing on cell wall pectic substances in relation to firmness of carrot tissue. *Food Chemistry*, 107, 1225-1235.

2.2 MATERIALS AND METHODS

2.2.1 Experimental setup

Carrot discs were subjected to three different treatments (80 °C/0.1 MPa, 100 °C/0.1 MPa, 80 °C/600 MPa) for varying time intervals. For the HP treatment, 80 °C was selected as this was the highest temperature feasible with the HP equipment available at the moment of the experiment. This treatment was compared with two thermal treatments at 0.1 MPa, one treatment at the same temperature (80 °C), a second treatment at a higher temperature (100 °C) at which the effects of thermal processing are more outspoken. After the treatments, the residual hardness and microstructural changes of the samples were evaluated. Alcohol insoluble residues (AIR) were prepared from the samples and sequentially extracted with water, CDTA and Na_2CO_3 solutions. These pectin fractions were analysed for GalA content, molecular mass (MM) distribution, DM, and neutral sugars (NS) content. A schematic overview of the experimental setup is presented in Figure 2.1. All chemicals, reagents and solvents used were of analytical grade.

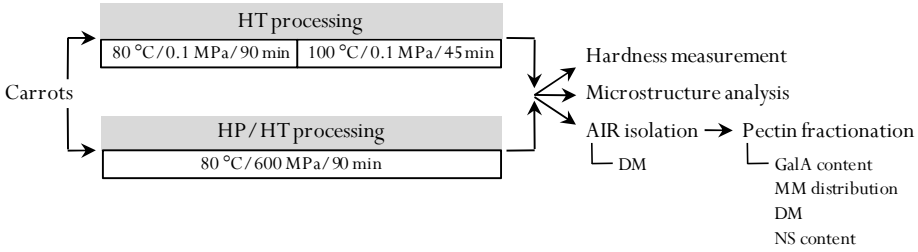


Figure 2.1: Schematic overview of the experimental setup.

2.2.2 Carrots

Carrots (*Daucus carota* cultivar Yukon, 2-3 cm diameter) were obtained from a local shop in Belgium and stored at 4 °C for a maximum period of one week before use. Discs (10 mm height and 12 mm diameter) were excised from the core of the carrots.

2.2.3 Thermal treatments

Carrot discs (10) encapsulated in stainless steel tubes (110 mm long, 13 mm internal diameter, and 1 mm thickness) filled with demineralised water as a brine (2.9 mL water/10 carrot discs) were subjected to thermal treatments (80 and 100 °C) in a thermostated oil bath. After a lag time of 5 min (experimentally determined by Smout *et al.* (2005)) a first sample (treatment time 0 min) was withdrawn. Subsequent samples were removed from the oil bath after preset time intervals. The samples were immediately cooled in an ice water bath and the

texture was determined. The brine solution was collected, frozen under liquid N_2 and stored at -40 °C for further analysis. Each sample consisted of 3 tubes and thus 30 carrot discs.

2.2.4 High-pressure/high-temperature treatments

HP/HT treatments were carried out in a custom-made laboratory scale HP unit (Resato) consisting of 6 individual vessels (6 x 43 mL, 20 mm internal diameter), each surrounded by a heating mantle containing water connected to a thermostat. The pressure transmitting medium consisted of a propylene glycol - water mixture (Resato PG fluid). This equipment allows processing conditions up to 800 MPa and 80 °C.

Each sample consisted of 10 carrot discs submerged in 2.9 mL demineralised water packed in a double-film polyethylene bag. The pressure vessels were equilibrated at the process temperature (80 °C). The time required to insert the samples and close the vessels was standardized at 2 min. Pressure was built up manually (till 600 MPa) using a slow pressurization rate (100 MPa/min) to minimize the temperature rise due to compression heating. After pressure build-up, an equilibration period of 5 min was taken into account to allow the temperature of the samples to evolve back to the desired temperature. The latter time point was considered as the starting time (treatment time 0 min) of the isothermal-isobaric treatment at 80 °C and 600 MPa. After preset time intervals, the individual vessels were decompressed instantaneously and the respective samples were removed from the vessels exactly 1 min later. Samples were immediately cooled in an ice water bath and the texture was determined. The brine solution was frozen under liquid N_2 and stored at -40 °C. Each sample consisted of 3 bags and thus 30 carrot discs.

2.2.5 Texture measurement

Texture is a multi-parameter attribute (Szczesniak, 2002) (see section 1.2). The parameter considered in this work is hardness. The carrot tissue hardness was evaluated by a compression test using a TA-XT2i Texture Analyser (Stable Micro Systems). The following parameters were used: load cell = 25 kg, probe = 25 mm diameter aluminium cylinder, and test speed = 1 mm/s (Sila *et al.*, 2004). The hardness of a carrot cylinder was defined as the maximum force needed to compress the carrot cylinder to 70% of its original thickness. The mean value of the compression forces of 26 carrot cylinders was considered as a single data point. After texture measurement, the carrot cylinders were frozen under liquid N_2 and stored at -40 °C.

2.2.6 Microstructure analysis

Carrot discs were cut axially in two pieces using a TA-XT2i Texture Analyser (Stable Micro Systems) equipped with a sharp knife blade which penetrated the

discs at a constant speed of 1 mm/s. Subsequently, the pieces obtained were cut (manually) transversely in slices of 2-3 mm thick. These slices were fixed using glutaraldehyde, followed by repeated washing and dehydration in a series of ethanol solutions (50, 70 and 95%). The dehydrated carrot slices were infiltrated with a Historesin Embedding Kit (Leica) for 5 days, polymerised and cut into 5 µm sections with a Microm HM355 microtome (Microm Laborgeräte GmbH). Sections were stained with 0.1% toluidin blue for 5 min followed by washing with water. Stained slices were examined with an Olympus BX-41 light microscope (Olympus, Optical Co. Ltd). Micrographs were taken using image analysis software (analySIS pro 5.0, Soft Imaging System GmbH).

2.2.7 Pectin methylesterase extraction and activity assay

Carrot cylinders were homogenised and mixed in 0.2 M TRIS-HCl pH 8.0 (TRIS = 2-amino-2-hydroxymethyl-1,3-propanediol) containing 1.0 M NaCl (1:1.3 w/v) for 2 h at 4 °C to extract cell wall-bound PME. The crude PME extract was obtained by filtration using a cheese cloth.

PME activity was determined by measuring the release of acid per time unit at pH 7.0 and 22 °C. The reaction mixture consisted of 2.0 mL crude extract and 30 mL of a 0.35% (w/v) apple pectin solution (DM 70 – 75%, Fluka) containing 0.117 M NaCl. During pectin hydrolysis, the pH was maintained at pH 7.0 by addition of 0.01 N NaOH using an automatic pH-stat titrator (Metrohm). One unit (U) of PME activity is defined as the amount of enzyme catalysing the hydrolysis of 1 µmol of methyl ester bonds per minute under aforementioned assay conditions.

2.2.8 Preparation of alcohol insoluble residue

Cell wall material was isolated as AIR as described by McFeeters and Armstrong (1984). Carrot tissue (10.0 g) was completely homogenised in 64 mL of 95% ethanol using a mixer (Buchi mixer B-400). The suspension was filtered and the residue was re-homogenised in 32 mL of 95% ethanol and filtered again. The residue was homogenised in 32 mL of acetone before final filtration followed by drying overnight at 40 °C. The AIR was ground using a mortar and pestle and stored in a desiccator.

2.2.9 Determination of pectin degree of methoxylation

The DM of carrot pectin was calculated as the ratio of moles of methoxyl groups to the moles of GalA residues. To determine the latter, pectin was hydrolysed with concentrated sulfuric acid according to the method of Ahmed and Labavitch (1977). Subsequently, the GalA content was determined colorimetrically as described by Blumenkrantz and Asboe-Hansen (1973). In this method, the hydrolysate is heated in concentrated sulfuric acid containing sodium borate and subsequently mixed with meta-hydroxy-diphenyl. A purple coloured product is

formed of which the absorbance is measured at 520 nm and 25 °C. The methoxyl content was estimated by hydrolysing the ester bonds of pectin with a NaOH solution (Ng and Waldron, 1997a) and colorimetric quantification of the released methanol as described by Klavons and Bennett (1986). Briefly, methanol is oxidised to formaldehyde by an alcohol oxidase and subsequently condensed with 2,4-pentanedione resulting in a coloured compound (3,5-diacetyl-1,4-dihydro-2,6-dimethylpyridine) of which the absorbance is measured at 412 nm and 25 °C. In both procedures, the respective hydrolyses were performed in duplicate, and 3 colorimetric analyses were carried out for each hydrolysate.

2.2.10 Pectin fractionation

Cell wall material was fractionated by extracting AIR sequentially with hot water, a solution of a chelating agent and an alkaline solution. AIR (0.25 g) was incubated, while stirring, in demineralised water (45 mL) for 5 min at 100 °C (Sila *et al.*, 2006b). The suspension was cooled under running tap water and filtered. The volume of the filtrate was adjusted to 50 mL (water soluble pectin, WSP). The residue was re-suspended in 45 mL of 0.05 M CDTA in 0.1 M potassium acetate pH 6.5 for 6 h at 28 °C (Chin *et al.*, 1999). Upon filtration, the volume of the filtrate was adjusted to 50 mL (chelator soluble pectin, CSP). The residue was re-incubated in 45 mL of 0.05 M Na_2CO_3 containing 0.02 M $NaBH_4$ for 16 h at 4 °C, and then for another 6 h at 28 °C (Chin *et al.*, 1999). The mixture was filtered and the volume of the filtrate was adjusted to 50 mL (Na_2CO_3 soluble pectin, NSP). Fractionations were performed in duplicate. All fractions were analysed for GalA content (as a measure for the pectin content) as described in section 2.2.9. As for all fractionations GalA content of both duplicates corresponded, further analyses were performed on pooled fractions. Fractions were lyophilised (Christ, Alpha 2-4), followed by determination of the DM (section 2.2.9), molecular mass distribution pattern and neutral sugars content.

2.2.11 Analysis of molecular mass distribution

Changes in MM distribution of carrot pectin during processing were studied using high-performance size exclusion chromatography (HPSEC). This was performed using an Äkta Purifier (GE Healthcare) equipped with a TSK-GEL $GMPW_{XL}$ column and a TSK-GEL PW_{XL} guard column (Tosoh Corporation). The pectin solution (20 µL) was injected and eluted for 25 min at 35 °C with 0.05 M $NaNO_3$ at a flow rate of 0.7 mL/min. The eluent was monitored using a Shodex RI-101 detector (Showa Denko K.K.). Deionised water (organic free, 18.2 MΩ·cm resistance) supplied by a Simplicity™ Millipore water purification system was used to prepare eluents and samples. Galacturonic acid was used daily to validate the system. Pullulan standards with MM ranging from 5900 to 788000 Da were eluted to check the MM range the column was able to separate. These 8 standards

eluted between 14.1 and 10.4 min; a log-linear relationship between MM and elution time was obtained. It should be kept in mind, however, that the hydrodynamic volumes of pullulan and pectin molecules with the same MM differ slightly. Hence, the standard curve only allows rough estimations of MM of pectins.

Lyophilised WSP and NSP samples dissolved in demineralised water (WSP: 0.25% w/v, NSP: 1.5% w/v) were extensively dialysed (MWCO 12000 - 14000 Da) against demineralised water. Dissolved CSP samples (5.0% w/v) were extensively dialysed against 0.1 M NaCl, followed by dialysis against demineralised water. Brine solutions were also dialysed against demineralised water. Samples were adjusted to a concentration of 0.05 M $NaNO_3$ before analysis.

2.2.12 Analysis of neutral sugars

High-performance anion exchange chromatography was carried out to analyse the neutral sugars content of the different pectin fractions. This was achieved using a Dionex BioLC system equipped with a quaternary gradient pump and a CarboPac PA1 column (Dionex). An ED50 electrochemical detector equipped with a gold electrode was used in the pulsed amperometric detection mode, performing a quadrupole potential waveform. The sample (25 µL) was injected and eluted (1.0 mL/min) in a gradient with 100 mM NaOH (A) and 1.0 M NaOAc (B) in deionised water (18.2 MΩ·cm) (C) as follows: 0→23 min, 15% A and 85% C; 23→35 min, linear gradient of 15→30% A, 0→30% B, 85→40% C; 35→45 min, linear gradient of 30→15% A, 30→0% B, 40→85% C. The column temperature was set at 30 °C. Commercial neutral sugar standards (L-rhamnose, L-arabinose, and D-galactose) at varying concentrations (1 – 5 ppm) were used for identification and quantification.

Sample preparation was as follows: 0.01 g of lyophilised pectin fraction was digested in 4.0 M trifluoroacetic acid (TFA) at 110 °C for 1.5 h. After cooling, the TFA was evaporated under vacuum at 40 °C for 40 min. The TFA-free samples were diluted with demineralised water before analysis.

2.3 RESULTS AND DISCUSSION

2.3.1 Hardness

Figure 2.2 illustrates the hardness of processed carrots relative to the hardness of raw carrots for the three different treatments. In all the cases, there was a rapid loss in the initial hardness due to membrane damage and turgor pressure loss (Greve *et al.*, 1994b; Basak and Ramaswamy, 1998). The hardness of the thermally treated carrots decreased further with increasing treatment time,

probably due to beta-eliminative degradation of pectin (Greve *et al.*, 1994a; Sila *et al.*, 2006b). In contrast, the HP/HT treated carrots did not undergo further softening, indicating that the beta-elimination reaction of pectin is inhibited at combined HP/HT treatments.

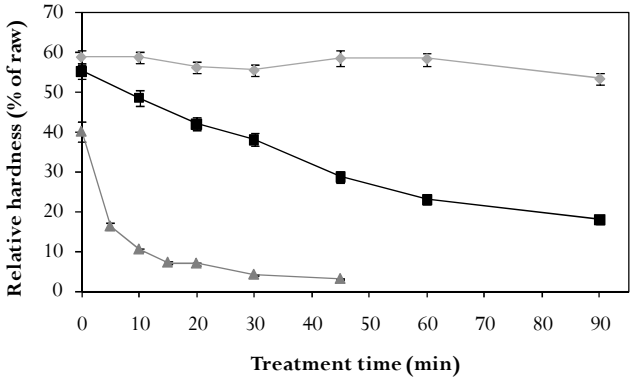


Figure 2.2: Changes in carrot discs' hardness during processing. ■ 80 °C/0.1 MPa, ▲ 100 °C/0.1 MPa, ◆ 80 °C/600 MPa. Hardness of treated carrots is expressed as a percentage of the hardness of raw carrots. Treatment time 0 min: start of the isothermal-isobaric treatment. Each point represents the mean value of 26 measurements (± standard error).

2.3.2 Microstructure

Figure 2.3 presents micrographs of carrot tissue. Raw carrot tissue showed well-defined, conspicuously well-stained cell walls (Figure 2.3 a). The intensity of the cell wall staining decreased progressively with increasing thermal impact accompanied by increased cell wall swelling and cell separation (Figures 2.3 b and c). These observations are probably due to heat induced solubilisation of the intercellular cementing pectin facilitating cell wall loosening (Waldron, 2004). In contrast, HP/HT treated tissue showed close resemblance to the raw tissue, indicating that pectin solubilisation was limited (Figure 2.3 d).

To determine tissue failure characteristics, micrographs were taken from the cutting edge (Figure 2.4). Tissue failure classically involves cell separation and/or cell breakage. If the forces holding the cells together are stronger than the cell walls, then failure will occur in the walls (cell breakage). If the forces holding the cells together are weaker than the cell walls, then cell separation will occur. Raw carrot tissue clearly showed cell breakage, indicating strong intercellular adhesion (Figure 2.4 a). In contrast, heat treated tissue showed cell separation (Figure 2.4 b). The results of the HP/HT treated tissues were intermediate, showing characteristics from both mechanisms (Figure 2.4 c).

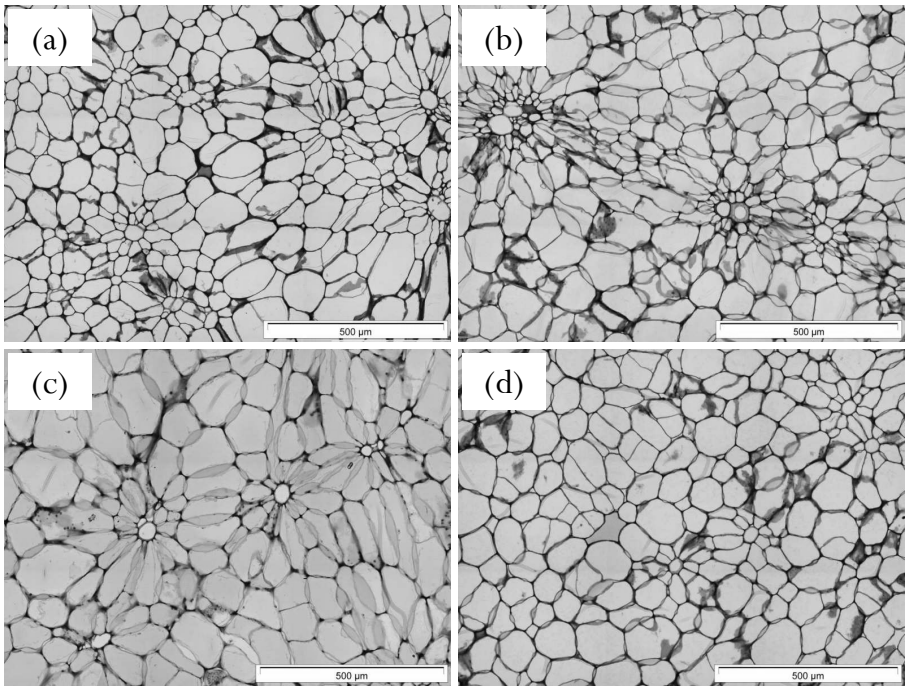


Figure 2.3: Micrographs of raw and processed carrot tissue, indicating changes in cell wall area. (a) raw carrots, (b) 80 °C/0.1 MPa/90 min, (c) 100 °C/0.1 MPa/45 min, (d) 80 °C/600 MPa/90 min.

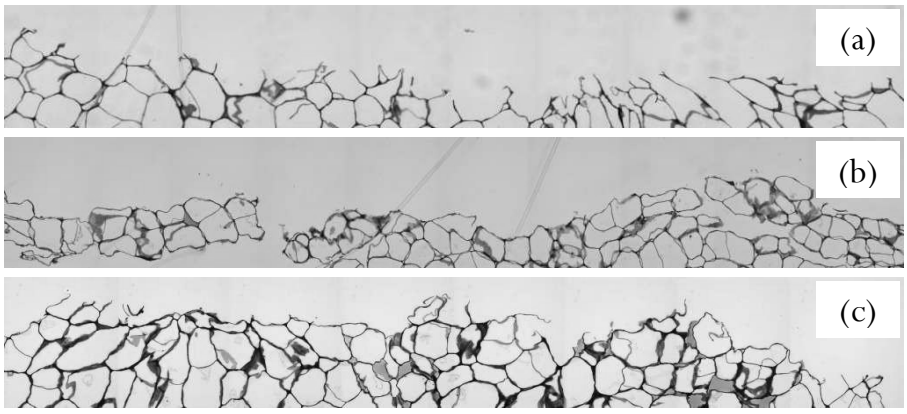


Figure 2.4: Micrographs of the cutting edge of raw and processed carrot tissue, indicating changes in tissue failure characteristics. (a) raw carrots, (b) 80 °C/0.1 MPa/45 min, (c) 80 °C/600 MPa/45 min.

2.3.3 Pectin structure

The macro- and microscopic observations clearly indicated different behaviour of carrot tissue during HT and HP/HT processing. In order to gain mechanistic insight, the cell wall material was isolated and the pectin structure analysed.

2.3.3.1 Degree of methoxylation of pectin

The DM of raw carrot pectin was estimated at 64%, which is in close agreement with the values reported in literature (DM = 60 – 69%) (Siliha *et al.*, 1996; Ng and Waldron, 1997a; Sila *et al.*, 2006a). For all treatment conditions studied, the DM decreased. However, HP/HT treatment showed a more pronounced reduction (Table 2.1). The decline may be explained by the occurrence of chemical demethoxylation of pectin at HT as reported by Sajjaanantakul *et al.* (1989). In case of the HP/HT treated samples, the high reduction may be explained by the combined effect of (a) chemical demethoxylation of pectin which is enhanced by pressurization (Verlent *et al.*, 2004) and (b) enzymatic demethoxylation of pectin by PME. It is established that carrot PME is a rather pressure-stable enzyme that is stabilised and activated by HP (Ly-Nguyen *et al.*, 2003; Sila *et al.*, 2007a; Jolie *et al.*, 2009). However, the latter has only been verified at milder p-T combinations than studied here. Ly-Nguyen *et al.* (2003) investigated the stability of purified carrot PME under mild heat (≤ 65 °C) and HP (≤ 825 MPa). Carrot PME was rather thermo-labile. Under isothermal-isobaric treatments, an antagonistic effect of temperature and pressure was observed at lower pressures (< 300 MPa) and higher temperatures (> 50 °C). Sila *et al.* (2007a) noticed a pronounced stimulation of the catalytic activity of carrot PME in model systems as well as in food systems (shredded carrots) with increasing temperature (< 55 °C) and pressure (≤ 500 MPa).

Table 2.1: Pectin DM (± standard deviation) of raw and processed carrots (thermally or HP/HT treated for different times).

	Degree of methoxylation (%)			
	0 min	20 min	45 min	90 min
80 °C/0.1 MPa	56.8 ± 2.1	52.0 ± 3.0	50.3 ± 2.3	52.7 ± 2.9
100 °C/0.1 MPa	57.7 ± 3.5	55.0 ± 3.2	52.9 ± 1.9	
80 °C/600 MPa	42.6 ± 3.5	37.4 ± 3.2	31.1 ± 2.0	34.2 ± 1.0
Raw	63.6 ± 5.3			

To verify whether the low pectin DM of the HP/HT treated carrots could indeed be linked to PME activity carrots were checked for residual PME activity. Raw carrots showed a PME activity of around 2679 U/kg. After 10 min treatment at 80 °C/0.1 MPa, no residual PME activity could be detected. However, after 10

min at 80 °C/600 MPa, residual PME activity (~ 398 U/kg) was noticed, even after 60 min treatment (~ 19 U/kg). This could be related to the stabilising effect of pressure on PME. Consequently, the reduction in DM of HP/HT samples could (partly) be due to PME activity. It was remarkable that after the equilibration time at 80 °C/600 MPa, the DM had already decreased from 64 to 43%. Sila *et al.* (2007a) also noticed an extensive decrease in DM of pressure/temperature treated carrots during pressure build-up and equilibration time (pressures up till 600 MPa in combination with temperatures up till 60 °C). In addition to favourable conditions for PME activity, enhanced contact between PME and pectin during pressurization could have been responsible for this phenomenon. As methyl esters are one of the main driving forces of the beta-elimination reaction, it is likely that the high reduction in DM of the HP/HT treated carrots triggered inhibition of the beta-elimination reaction, consequently preventing softening. In addition, cell adhesion might have been enhanced by cross-linking of the demethoxylated pectin by endogenous Ca^{2+}. Intracellular Ca^{2+} might have been released from the cells due to pressure-induced membrane permeabilization to become available for binding to pectin.

2.3.3.2 Changes in pectin solubility

The AIR was fractionated based on its solubility in different solvents. Raw carrots contained predominantly WSP, a substantial amount of CSP, and a low amount of NSP. During processing, these pectin amounts changed strikingly (Table 2.2). Thermally treated carrots were characterized by an increasing amount of WSP with increasing treatment time paralleled by a decreasing amount of CSP and NSP. These results are in accordance with the results found in literature (Ng and Waldron, 1997a; Sila *et al.*, 2006a; Sila *et al.*, 2006b) and indicate that a substantial degradation and solubilisation of pectin occurs during thermal processing. For the HP/HT treated carrots, at treatment time 0 min, a low amount of WSP was noticed while the other fractions were proportionally higher. During HP/HT processing, the pectin fractions hardly changed, corresponding with the unaltered hardness of the carrots. The low amount of WSP and high amount of CSP of HP/HT treated carrots could be explained by increased ionic cross-linking as a result of the reduced DM. One possible explanation for the high amount of NSP could be that under HP/HT conditions particular (Na_2CO_3 soluble) ester bonds are formed. Another possible explanation could be that pectin with a very low DM, and consequently high degree of polymerisation (because no or limited beta-elimination occurred), is very strongly ionically bound to the cell wall so that it could not be released with the CDTA solution applied.

To evaluate the pectin fractionation, the amount of GalA found in AIR was compared to the amount of GalA found in WSP, CSP and NSP. The extraction yield of the pectin fractionation for the carrots treated at 80 °C and 0.1 MPa for

20 min was 81% (Table 2.3). Interestingly, the extraction yields for the carrots treated at 80 °C and 600 MPa were clearly lower, ranging from 53 to 70%. To account for the difference, it is possible that there was loss of GalA or that some pectin remained attached to the fractionation residue. Therefore, the GalA content of the fractionation residue was determined. From Table 2.3 it is obvious that a high amount of GalA remained associated to the residue of the HP/HT treated carrots, indicating that a part of the pectin strongly interacted with the other cell wall polymers that it could not be solubilised with the procedure used. These differences in pectin solubility are a probable explanation for the differences in textural characteristics of the samples.

2.3.3.3 Molecular mass distribution of pectin fractions

MM distributions of pectin fractions were monitored by HPSEC (Figure 2.5). The elution profile of WSP of raw carrots shows two unresolved peaks, a small high MM peak (elution time ~ 10.5 min, equivalent MM ~ 690 kDa) coinciding with the elution profile of the CSP (results not shown) and NSP fractions and a larger peak (elution time ~ 12 min) originating from pectins with a lower MM (equivalent MM ~ 85 kDa). During thermal processing, distinct changes in polymer concentrations and MM distribution patterns were apparent; being most pronounced at 100 °C. With thermal progression, two main changes occurred in the WSP fraction (Figure 2.5 left). First, the area of the early eluting peak increased with increasing processing time. This represents the proportions of the original CSP and NSP fractions which were thermosolubilised, becoming water soluble. Secondly, there was a shift in the later eluting peak to lower MM, illustrating transformations in the WSP fraction due to depolymerisation. This clearly demonstrated a dynamic change in pectin fractions during thermal processing, and corresponds with the increasing concentrations of WSP (Table 2.2). A complementary trend in changes in MM distribution was observed in the NSP fraction (Figure 2.5 right). Unlike what could be observed in the WSP fraction, there was no clear shift toward low MM polymers. However, decreasing quantities of homogeneously distributed polymers became evident with increasing thermal severity. Contrary, HP/HT processing led to different MM patterns. The elution profile of the WSP fraction showed only one peak, which neither clearly increased in area nor shifted towards lower MM, indicating that less thermosolubilisation occurred.

MM distributions of the matrix polymers in the brine solution were also analysed (Figure 2.6). With increasing thermal processing time the polymer concentration of the brine increased. Furthermore, the polymers occupied a broader MM range, indicating extensive pectin solubilisation. In contrast, the brine solution associated with the HP/HT processed carrots contained almost no matrix polymers, indicating less solubilisation.

Table 2.2: GalA content (mg GalA/g AIR ± standard deviation) of pectin fractions (WSP, CSP, NSP) extracted from AIR of raw and processed carrots (thermally or HP/HT treated for different times).

	GalA content of carrot pectin fractions (mg GalA/g AIR)											
	0 min			20 min			45 min			90 min		
	WSP	CSP	NSP	WSP	CSP	NSP	WSP	CSP	NSP	WSP	CSP	NSP
80 °C/0.1 MPa	100.6 ± 4.7	73.0 ± 3.6	25.3 ± 0.9	112.6 ± 1.8	64.0 ± 4.1	22.7 ± 0.7	117.2 ± 5.6	70.6 ± 0.4	27.4 ± 0.9	119.9 ± 1.7	68.3 ± 1.4	21.6 ± 2.0
100 °C/0.1 MPa	128.7 ± 3.4	64.3 ± 0.7	25.1 ± 0.9	132.0 ± 3.6	54.7 ± 0.8	10.1 ± 0.5	136.3 ± 3.5	52.1 ± 1.0	7.1 ± 0.1			
80 °C/600 MPa	23.7 ± 0.8	72.5 ± 1.3	64.4 ± 3.1	15.1 ± 0.7	60.2 ± 3.9	56.0 ± 0.9	22.4 ± 1.1	86.2 ± 1.3	75.0 ± 3.2	27.8 ± 1.0	81.0 ± 1.3	62.8 ± 1.7
Raw	127.7 ± 4.4	54.4 ± 0.9	24.9 ± 0.7									

Table 2.3: GalA content (mg GalA/g AIR) of AIR, pectin fractions and residue, and GalA extraction yields (%) of pectin fractionation.

	GalA content (mg GalA/g AIR)			Yield (%)	
	AIR	WSP+CSP+NSP	Residue	Yield without residue[a]	Yield with residue[b]
80 °C/0.1 MPa/20 min	245.7	199.3	6.4	81	84
80 °C/600 MPa/20 min	247.5	131.3	46.5	53	72
80 °C/600 MPa/45 min	263.6	183.6	40.1	70	85
80 °C/600 MPa/90 min	267.6	171.6	24.1	64	73

[a] Yield taking into account GalA content of WSP, CSP and NSP.

[b] Yield taking into account GalA content of WSP, CSP, NSP and residue.

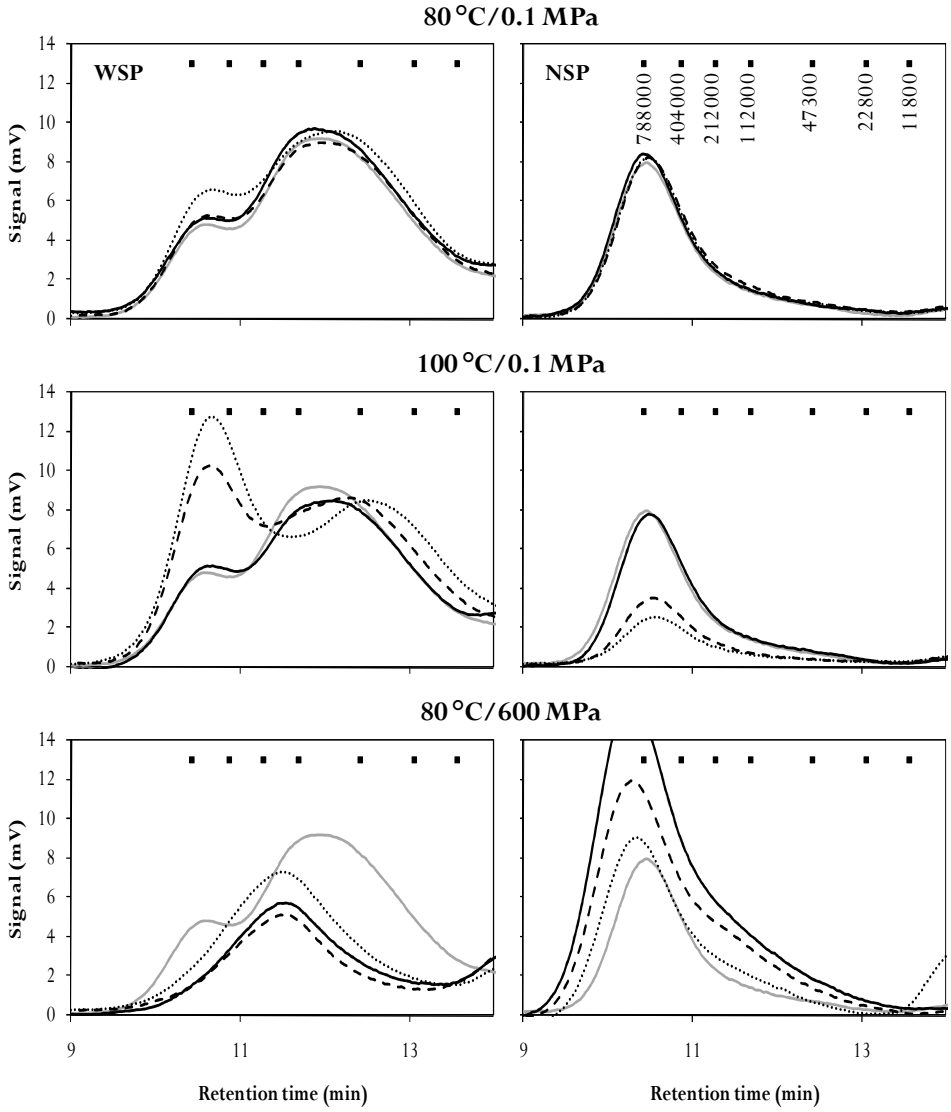


Figure 2.5: MM distribution of WSP (left) and NSP (right) of raw (grey line) and processed carrots (treatment time 0 min: black line, 20 min: dashed line, 45 min (100 °C) or 90 min (80 °C): dotted line). ■ Elution times of pullulan standards (MM (Da) indicated in upper right graph).

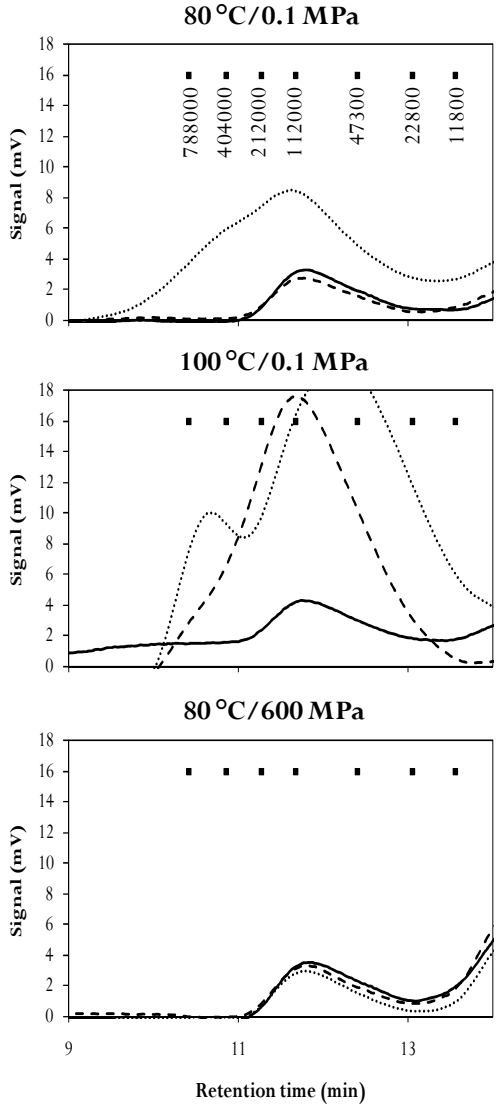


Figure 2.6: MM distribution of solubilised matrix polymers in brine from processed carrots (treatment time 0 min: black line, 20 min: dashed line, 45 min (100 °C) or 90 min (80 °C): dotted line). ■ Elution times of pullulan standards (MM (Da) indicated in upper graph).

2.3.3.4 Degree of methoxylation of WSP and CSP

In addition to changes in GalA concentrations, variations in the DM of the pectin fractions were observed (Table 2.4). WSP was highly methoxylated with a DM in the range 50 – 82%. In contrast, CSP was lowly methoxylated with a DM in the range 21 – 40%. The trend was similar to the one observed in the DM of the AIR: with increasing processing time the DM decreased. The DM of the NSP fraction could not be determined because the alkaline saponification procedure used in extraction removes its methoxyl groups.

Table 2.4: DM (± standard deviation) of WSP and CSP extracted from AIR of raw and processed carrots (thermally or HP/HT treated for different times).

	Degree of methoxylation (%)			
	0 min	20 min	45 min	90 min
WSP				
80 °C/0.1 MPa	77.8 ± 5.1	70.3 ± 2.0	n.d.	69.0 ± 5.9
100 °C/0.1 MPa	69.0 ± 3.7	63.4 ± 4.4	59.0 ± 2.2	
80 °C/600 MPa	53.7 ± 1.3	50.5 ± 4.2	50.0 ± 1.2	58.9 ± 1.4
Raw	81.8 ± 2.4			
CSP				
80 °C/0.1 MPa	37.7 ± 1.6	33.1 ± 2.7	34.2 ± 1.0	32.8 ± 0.8
100 °C/0.1 MPa	30.7 ± 4.8	27.9 ± 1.0	21.5 ± 0.9	
80 °C/600 MPa	39.7 ± 1.0	32.1 ± 3.1	26.4 ± 0.8	36.6 ± 1.0
Raw	36.3 ± 0.9			

n.d.: not determined

2.3.3.5 Neutral sugars content of pectin fractions

Pectin (in particular the "hairy" regions) contains many different neutral sugars of which Rha, Ara and Gal are the most abundant ones. These sugars could be identified and quantified using anion exchange chromatography combined with pulsed amperometric detection.

Table 2.5 shows the levels of the main pectin monosaccharides present in the different pectin fractions of raw carrots. The CSP and NSP fractions were found to have lower levels of neutral sugars than the WSP fraction. However, it should be noted that the lyophilised CSP and NSP fractions contained salts, thus influencing the absolute pectin amounts weighed per gram. Overall, Gal was the most abundant neutral sugar, followed by Ara and than Rha. To obtain an idea of the pectin composition of the different pectin fractions, the ratio of GalA to neutral sugars was calculated. It is obvious that the NSP fraction is mainly made up of neutral sugar rich rhamnogalacturon (RG-I and RG-II) whereas the WSP

and CSP fractions are relatively enriched with HG. Together with the results of section 2.3.3.4, it can be concluded that WSP contains the highly methoxylated HG whereas CSP the lowly methoxylated HG.

Table 2.5: Sugar content of lyophilised pectin fractions of raw carrots (mg/g solid ± standard deviation).

	Sugar content (mg/g solid)				
	Rha	Ara	Gal	GalA	GalA:NS
WSP	9.60 ± 1.07	38.41 ± 0.95	68.68 ± 1.82	270.12 ± 6.17	2.32 ± 0.04
CSP	0.30 ± 0.06	1.14 ± 0.10	1.20 ± 0.68	6.80 ± 0.09	2.71 ± 0.86
NSP	2.01 ± 0.47	7.34 ± 1.53	13.97 ± 4.68	7.99 ± 0.96	0.36 ± 0.10

NS: Rha+Ara+Gal

The trend observed for the changes in neutral sugars content of the fractions during processing complemented the changes in pectin solubility discussed in section 2.3.3.2. The concentration of pectin-related neutral sugars increased with increasing thermal processing time in the WSP fraction, while it decreased in the NSP fraction (results not shown). These results suggest a considerable thermal fragmentation of pectin hairy regions; an indication that part of the pectin solubilised contained high levels of side chains (and not only HG regions). This is in contrast with the results obtained by Ng and Waldron (1997a) and Stolle-Smits *et al.* (1997) who observed an increase in the GalA to NS ratio of WSP. As can be seen from Figure 2.6, a large amount of polymers leached out into the brine during processing. Possibly, these very soluble polymers were enriched in GalA rich polysaccharide moieties. In the pectin fractions of the HP/HT treated carrots, the neutral sugars amount remained roughly constant during processing; confirming the stability of these fractions.

2.4 CONCLUSION

During both HT and HP/HT processes carrots showed an initial texture loss, probably due to loss of turgor pressure. In contrast to thermally treated carrots which underwent further softening with treatment time, HP treated carrots did not.

Texture loss of thermally treated carrots can be explained in terms of the beta-eliminative depolymerisation of pectin. Therefore, texture preservation of HP/HT treated carrots could be explained by inhibition of the beta-elimination reaction. Two possible explanations emerge. On the one hand, it is possible that pressure directly inhibits the beta-elimination reaction. On the other hand, it is possible that the low DM of the respective samples triggered inhibition of the

beta-elimination. Moreover, the lowly methoxylated pectin could have formed fortifying networks with Ca^{2+} present. However, other pressure-induced mechanisms might be playing a role. The HP/HT treated carrots contained a low WSP content as opposed to the CSP and NSP content, and a substantial amount of pectin remained in the fractionation residue. Conversion of WSP in CSP could be explained by the extensive demethoxylation. There were no clear explanations found for the increase in GalA content of the NSP fraction and the fractionation residue.

In summary, regarding texture preservation of processed fruits and vegetables, the combination of HPP and elevated temperature proofed to be advantageous, making further research including stronger p-T processing conditions worthwhile.

3 EFFECT OF HIGH-PRESSURE/HIGH-TEMPERATURE PROCESSING ON CHEMICAL PECTIN CONVERSIONS[2]

3.1 INTRODUCTION

Because of the complex composition of real food systems and the many factors playing during processing, it is difficult to find causal relationships between processing and textural changes of fruits and vegetables. Insight can be gained in different ways (Kunzek *et al.*, 1999). In the previous chapter, cell wall material from processed food systems was isolated and analysed. Another approach is to use pectin model systems with well-known composition to investigate which reactions are occurring during processing.

As in the previous chapter inhibition of the beta-elimination reaction by pressure was put forward as a possible explanation for the observed textural differences, this chapter aimed at a better understanding of the effect of HP/HT processing at the molecular level. The effect of HP/HT processing on the beta-eliminative depolymerisation was investigated in pectin model systems. Likewise, the effect on the chemical demethoxylation was studied, as pectin demethoxylation influences the beta-elimination reaction rate (see section 1.2.2.1). The selected processing conditions were more intense than those used in the previous chapter in order to include HP sterilization conditions.

[2] This chapter is based on the following paper:
De Roeck, A., Duvetter, T., Fraeye, I., Van der Plancken, I., Sila, D.N., Van Loey, A., Hendrickx, M. (2009). Effect of high-pressure/high-temperature processing on chemical pectin conversions in relation to fruit and vegetable texture. *Food Chemistry,* 115, 207-213.

3.2 MATERIALS AND METHODS

3.2.1 Experimental setup

Apple pectin solutions at pH 6.5 were on the one hand treated at HT and 0.1 MPa, on the other hand at HT in combination with HP for varying time intervals. Subsequently, the extent of beta-elimination and demethoxylation was determined spectrophotometrically by measuring the unsaturated uronides and methanol formed. A zero-order reaction model was selected to fit the data. A schematic overview of the experimental setup is presented in Figure 3.1. All chemicals used were of analytical grade.

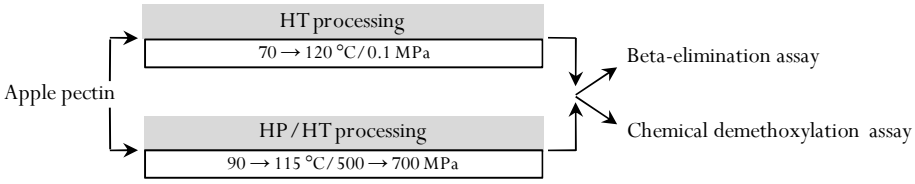


Figure 3.1: Schematic overview of the experimental setup.

3.2.2 Pectin

Water soluble apple pectin (MM = 30 – 100 kDa, DM = 70 – 75%, Fluka) was used for all experiments. The sugar composition of this apple pectin was determined by Fraeye *et al.* (2007b).

3.2.3 Thermal treatments

A 0.3% (w/v) solution of apple pectin in 0.1 M Na-phosphate buffer pH 6.5, divided over screw-capped test tubes (2.0 mL per tube), was heated at 70, 80, 90, 100, 110 or 120 °C in a thermostated oil bath. After an equilibration period of 5 min, allowing the solution to reach the desired temperature, a first sample (treatment time 0 min) was withdrawn. Subsequent samples were removed after preset holding times. The samples were immediately cooled in an ice water bath and analysed for unsaturated uronides and methanol content.

3.2.4 High-pressure/high-temperature treatments

A 0.3% (w/v) pectin solution in 0.1 M MES-NaOH pH 6.5 (MES = 2-(N-morpholino)ethanesulfonic acid) was divided over flexible microtubes (500 µL), and subsequently treated at 90, 110 and 115 °C in combination with 500, 600 and 700 MPa for different time intervals.

The HP/HT treatments were carried out in the same HP unit as mentioned in the previous chapter. However, at this time, the equipment was adapted to operate at temperatures up to 120 °C. Therefore, the heating mantle was replaced by a

heating coil containing silicon oil and a new external HP intensifier was installed allowing faster pressure build-up. Moreover, the equipment was computerized allowing computer-controlled pressure build-up and data logging of both pressure and temperature.

First, a protocol had to be developed to treat samples in a reproducible way at semi-constant HP/HT. As an example, in Figure 3.2 the temperature and pressure history of a pectin sample during treatment at 110 °C and 600 MPa is shown (starting from pressure build-up). The samples were inserted in cylindrical, polyoxymethylene acetal (POM) sample holders (85 mm long, 12 mm internal diameter, and 3 mm thickness), which were filled with water (excluding air) and closed with a movable stopper. These sample holders were tailor-made to fill the vessels optimally, so the ratio of sample volume to pressure medium volume is as large as possible and constant. The sample holders (containing the samples) were equilibrated at room temperature. Subsequently, the sample holders were transferred to the pressure vessels already equilibrated at the final process temperature (110 °C). The vessels were closed and the temperature in the sample holder was allowed to rise to an initial temperature (e.g. ~ 69 °C) which was dependent on the desired process temperature after pressure build-up. This preheating (t', Figure 3.2) was the result of heat transfer from the pressure medium to the samples. This initial temperature was beforehand experimentally determined for each p-T combination under study. Sample holders with a 1.6 mm hole were used to allow temperature measurement inside the sample holder using a 36.8 mm type J thermocouple attached to the pressure vessel stopper. Subsequently, pressure was built up very fast (t''); increasing in 5 s from 0.1 to 150 MPa and then from 150 MPa to the set pressure (600 MPa) at a rate of 10 MPa/s. This was accompanied by a temperature rise (e.g. up to ~ 107 °C) due to compression heating. After attaining the desired pressure, 10 s (t''') were counted before the valves of the individual vessels were closed. During pressure holding the temperature of the sample continued to increase up to 110 °C due to the higher temperature of the pressure medium. A constant temperature was reached within 1 min. It was preferred to attain a somewhat lower temperature than the process temperature at the end of pressure build-up, to avoid a temperature overshoot. After preset holding times (t_2) the individual vessels were decompressed instantaneously by opening the respective valves. Exactly 30 s after pressure release the samples were removed from the pressure vessels and cooled in an ice water bath. Subsequently, the samples were analysed for unsaturated uronides and methanol content. In Figure 3.2 the temperature and pressure evolution of a sample during two independent runs is represented, illustrating the reproducibility of the method.

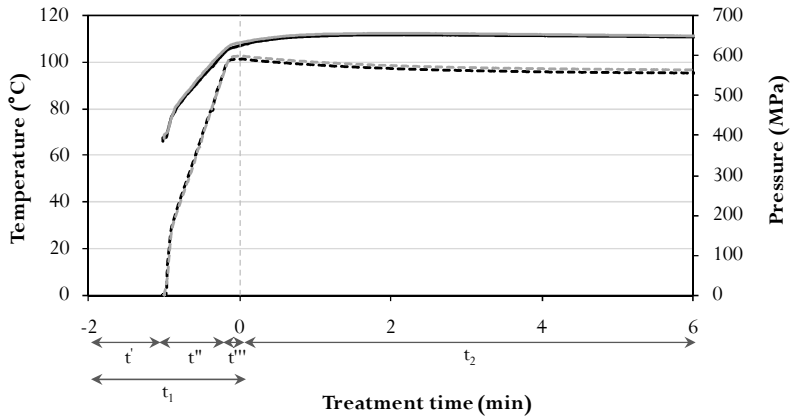


Figure 3.2: Temperature (full lines) and pressure (dashed lines) history of a pectin sample during treatment at 110 °C and 600 MPa, starting from pressure build-up (black lines: first run; grey lines: second run). t', t'', t''' are the time of preheating, pressure build-up, and equilibration. t_1 is the total preprocess time, t_2 the holding time.

3.2.5 Determination of unsaturated uronides

The extent of beta-elimination was monitored by measuring the formation of unsaturated uronides during treatment, as indicated by absorbance changes at 235 nm. The concentration of unsaturated uronides was calculated using an average molar extinction coefficient of 5412 $M^{-1}\cdot cm^{-1}$ (Sajjaanantakul *et al.*, 1989).

3.2.6 Determination of methanol and pectin DM

The amount of methanol released during treatment and the pectin DM were determined as described in section 2.2.9.

3.2.7 Data analysis

Demethoxylation and beta-elimination kinetic parameters were obtained using a two-step regression approach. First, the reaction rate constant k at a given temperature and pressure was determined by plotting the concentration of either methanol or unsaturated uronides as a function of time. Taking into account only the initial linear part of the curve, both reactions could be adequately modelled by pseudo zero-order reaction kinetics (Fraeye *et al.*, 2007a):

$$\frac{\partial C}{\partial t} = k$$

which can be integrated at isothermal-isobaric conditions (k time independent) to

$$C_t = C_0 + kt$$

with C_0 the concentration of methanol or unsaturated uronides at treatment time 0 min, C_t the concentration at treatment time t (min), and k the reaction rate constant (mM·min^{-1}).

In the second step, the temperature and pressure dependence of the reaction rate constants was determined. The temperature dependence of the rate constant at constant pressure is expressed in terms of the activation energy E_a and estimated using the Arrhenius equation:

$$k_T = k_{ref} exp\left[\frac{E_a}{R_g}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right]$$

where E_a is the activation energy (J·mol^{-1}), k_T the reaction rate constant (mM·min^{-1}) at temperature T (K), k_{ref} the reaction rate constant (mM·min^{-1}) at reference temperature T_{ref} (K), and R_g the universal gas constant (8.314 J·mol^{-1}·K^{-1}). The E_a values were estimated by plotting the natural logarithm of the k value against the reciprocal of the respective absolute temperature. The pressure dependence of the rate constant at constant temperature is expressed in terms of the activation volume V_a and calculated using the Eyring concept:

$$k_p = k_{ref} exp\left[\frac{V_a}{R_g T}(p_{ref} - p)\right]$$

where V_a is the activation volume (cm^3·mol^{-1}), k_p the reaction rate constant (mM·min^{-1}) at pressure p (MPa), k_{ref} the reaction rate constant (mM·min^{-1}) at reference pressure p_{ref} (MPa), and R_g the universal gas constant (8.314 cm^3·MPa·mol^{-1}·K^{-1}). The V_a values were estimated by plotting the natural logarithm of the k value against pressure. A positive E_a or negative V_a value indicates that the reaction rate constant increases with respectively increasing temperature or pressure. A high absolute value of E_a or V_a signifies that the rate constant depends strongly on respectively temperature or pressure.

3.2.8 Calculation of pH under high-pressure and high-temperature

Both the extreme pressures and temperatures involved in HP/HT treatments alter the pK_a of weak acids and bases within samples. This combined pressure and temperature effect can result in a reversible but significant pH shift in samples undergoing HP/HT treatment. Therefore, to evaluate whether the observed phenomena were directly caused by temperature and pressure changes, or also by pH changes, the pH of MES at the different HP/HT conditions examined was calculated according to the method of Bruins *et al.* (2007). For the calculation, the pH at atmospheric pressure, the reaction volume ΔV^0, the pK_a^0, and the ΔpK_a^0/°C of the buffer must be known. The superscript 0 denotes the value at atmospheric pressure. The pK_a^0 and ΔpK_a^0/°C can easily be found in text books

(e.g. Dawson *et al.* (1969)). Kitamura and Itoh (1987) have measured reaction volumes for many protonic ionisation reactions. First, the shift in pK_a due to the HT and HP has to be calculated. The pK_a at HT can be obtained using $\Delta pK_a^0/°C$. The following relation described by El'yanov and Hamann (1975) was used to compute the pK_a at HP:

$$ln\left(\frac{K_a}{K_a^0}\right) = -\frac{p\Delta V^0}{R_g T(1+bp)}$$

where p denotes pressure (MPa), R_g the universal gas constant (8.314 $cm^3 \cdot MPa \cdot mol^{-1} \cdot K^{-1}$), T the absolute temperature (K), and b a constant ($9.2 \cdot 10^{-4}$ MPa^{-1}). Irrespective of the parameter for which is corrected first, basic assumptions have to be made. In case the pK_a is corrected first for temperature, it is assumed that the ΔV^0 does not change with temperature. In the other case, it is believed that the $\Delta pK_a^0/°C$ does not change with pressure. The obtained K_a can then be used with the following equation:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

As this equation has too many unknowns to be solved, the molarity of the buffer solution and a mass balance on the hydrogen atoms can be used as boundary conditions. The total amount of hydrogen atoms should be the same under HP and HT as under atmospheric pressure and room temperature. Finally, the $[H^+]$, and thus the pH can be calculated by iteration.

The calculations do not take into account the buffering capacity of the pectin itself, due to its many carboxyl groups. However, since the pK_a of GalA (the main building block of pectin) is 3.5, the buffering capacity of pectin at pH 6.5 will be rather limited. Nevertheless, it is not known in which way this pK_a is influenced by temperature and pressure.

3.3 RESULTS AND DISCUSSION

3.3.1 Influence of temperature on the rate of pectin degradation

A pectin solution in 0.1 M Na-phosphate buffer pH 6.5 was subjected to temperatures ranging from 70 to 120 °C at 0.1 MPa and the rate of pectin demethoxylation and beta-elimination was monitored (Figure 3.3). A pH of 6.5 was chosen as this is a pH value relevant for many vegetables, as well as a pH where demethoxylation and beta-elimination occur at a significant rate. A phosphate buffer was selected because this buffer is, with a $\Delta pK_a^0/°C$ of -0.003, little temperature dependent. Table 3.1 shows for the different temperatures the

theoretical pH of the solution and the reaction rate constants of the pectin conversions studied.

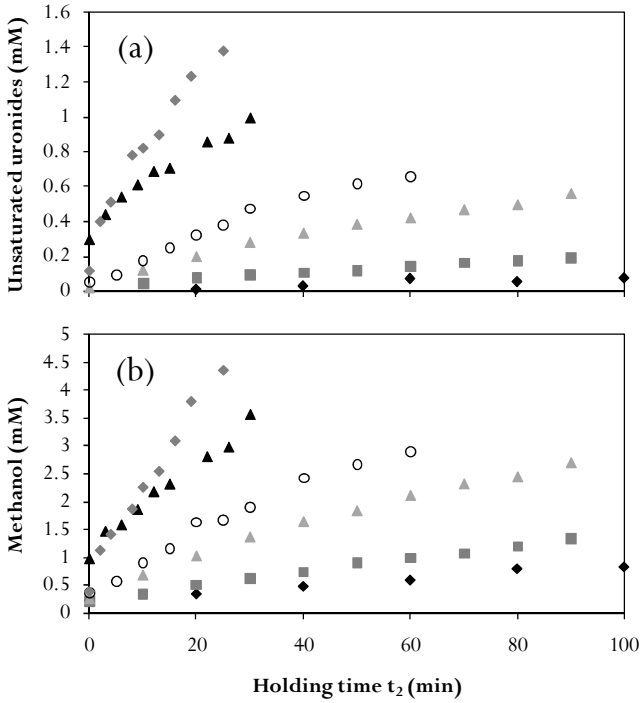


Figure 3.3: Formation of unsaturated uronides (a) and methanol (b) as a function of holding time when incubating pectin dissolved in 0.1 M phosphate buffer pH 6.5 at different temperatures and 0.1 MPa. ◆ 70 °C, ■ 80 °C, ▲ 90 °C, ○ 100 °C, ▲ 110 °C, ◆ 120 °C.

Rate constants of both reactions increased with increasing temperature. The temperature dependence of the reaction rate constants could be adequately modelled with the Arrhenius equation, resulting in activation energies of 96.6 kJ/mol and 70.8 kJ/mol, respectively for beta-elimination and demethoxylation. The higher E_a value for beta-elimination implies that any temperature rise results in a stronger acceleration of beta-elimination than of demethoxylation. Fraeye *et al.* (2007a) estimated similar activation energies, ranging from 80.2 to 123.9 kJ/mol for beta-elimination and from 44.4 to 96.9 kJ/mol for demethoxylation, for apple pectin with different DMs at various pH values. The ratio of the rate constant for demethoxylation (k_d) to the rate constant for beta-elimination (k_β) was calculated for the different temperatures. A ratio k_d/k_β from 2 to 9 was observed, indicating that, at all temperatures, demethoxylation was faster than beta-elimination. The ratio decreased with increasing temperature,

corresponding with the observation that the activation energy of beta-elimination is higher than that of demethoxylation.

Table 3.1: Theoretical pH values and experimentally determined zero-order reaction rate constants and activation energies (± standard error of regression) for beta-elimination and demethoxylation of pectin treated in phosphate buffer at different temperatures and 0.1 MPa.

T (°C)	pH	beta-elimination k_β (10^{-6} M·min^{-1})	demethoxylation k_d (10^{-6} M·min^{-1})
70	6.37	0.69 ± 0.07	5.63 ± 0.27
80	6.34	1.81 ± 0.07	12.37 ± 0.31
90	6.31	7.83 ± 0.49	30.30 ± 1.57
100	6.28	14.70 ± 0.38	51.71 ± 3.45
110	6.25	29.95 ± 2.65	80.47 ± 6.74
120	6.22	45.03 ± 4.73	133.84 ± 9.64
	E_a (kJ·mol^{-1})	96.6 ± 7.8	70.8 ± 3.8

3.3.2 Influence of high-temperature in combination with high-pressure on the rate of pectin degradation

A MES buffer was chosen for combined HP/HT experiments. Amine buffers such as MES and TRIS are less sensitive to pressure ($\Delta V^0 \sim 4$ cm^3/mol) than anionic buffers such as phosphate and citrate ($\Delta V^0 \sim$ -10 to -20 cm^3/mol). Within the pressure-tolerant buffers MES, with a ΔpK_a^0/°C of -0.011, is also least sensitive to temperature. In comparison, the ΔpK_a^0/°C of TRIS is -0.028. In Figure 3.4 the estimated shift in pH of MES within the p-T window examined is illustrated. As the pK_a is first corrected for temperature and then for pressure, it is assumed that ΔV^0 does not change with temperature. For the different HP/HT combinations, the pH values are ranging from 5.69 to 6.02. If the pK_a is first corrected for pressure and afterwards for temperature (assuming ΔpK_a^0/°C does not change with pressure) the pH estimations differ slightly, ranging from 5.74 to 6.08 (results not shown). The higher the temperature is, the lower the pH value. Conversely, applying HP increases the pH.

The pectin solution was treated in a p-T window of 500 – 700 MPa and 90 – 115 °C. The formation of unsaturated uronides and methanol at 110 °C is shown in Figure 3.5. Similar trends were observed at 90 and 115 °C. At 0.1 MPa, both beta-elimination and demethoxylation occurred at a significant rate. However, the rate constants, 14.62·10^{-6} M/min for beta-elimination and 39.64·10^{-6} M/min for demethoxylation, were remarkably lower as compared with treatment in phosphate buffer (Table 3.1). A possible explanation could be the more pronounced temperature dependence of MES resulting in a pH of 5.57 at 110 °C

(Figure 3.4). However, differences in ion concentrations could also play a role as it has already been described that the beta-elimination rate is affected by ions (Sajjaanantakul *et al.*, 1993) (see section 1.2.2.1). HP decelerated or even inhibited the beta-elimination reaction, whereas it had a pronounced stimulating effect on the demethoxylation. This stimulating effect has already been observed by Verlent *et al.* (2004) and can be explained based on the principle of Le Chatelier. As the curves were horizontal or levelled off quite rapidly, kinetic parameters (k, V_a) were difficult to estimate.

It is difficult to judge whether the inhibition of the beta-elimination is due to a direct effect of pressure, or if other, indirect effects of the HP/HT treatment play a role. If the beta-elimination is accompanied by an increase in reaction volume, direct inhibition of beta-elimination by HP could be explained by the principle of Le Chatelier. However, other or additional mechanisms (i.e. changes in pH and/or DM during treatment) could be responsible for inhibition. They are discussed in the following two paragraphs.

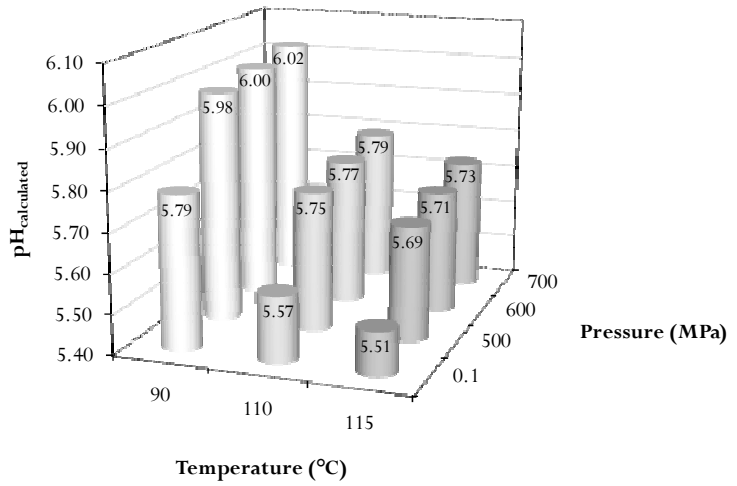


Figure 3.4: Calculated pH of 0.1 M MES buffer pH 6.5 (at 0.1 MPa and 25 °C) under the assumption that ΔV^0 does not change with temperature.

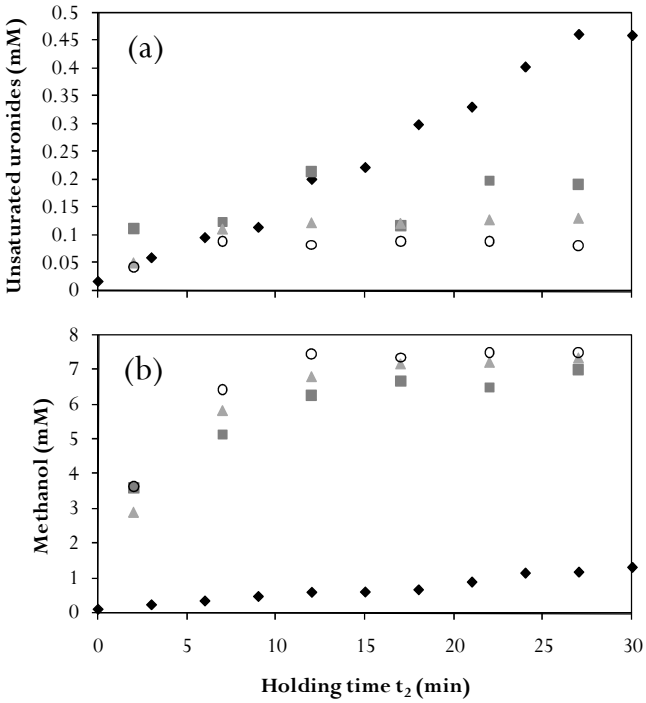


Figure 3.5: Formation of unsaturated uronides (a) and methanol (b) as a function of holding time when incubating pectin dissolved in 0.1 M MES pH 6.5 at different pressure levels and 110 °C. ◆ 0.1 MPa, ■ 500 MPa, ▲ 600 MPa, ○ 700 MPa.

3.3.3 Influence of pH on the rate of pectin degradation

The effect of pH on the beta-elimination and demethoxylation was investigated at 0.1 MPa. Pectin solutions in 0.1 M citrate buffer at pH values from 6.2 to 4.5 were treated at 100 °C and 0.1 MPa (Figure 3.6). Decreasing the pH decelerates both beta-elimination and demethoxylation, as also observed by Fraeye *et al.* (2007a). At pH 4.5, both reactions hardly occurred.

As previously mentioned (section 3.2.8), the pH of samples undergoing HP/HT treatment can shift. If this shift results in a lowering of the pH, deceleration or inhibition of the beta-elimination can occur. According to the pH calculations (Figure 3.4) the pH values during pressure treatment of MES buffer at 110 °C are ranging from 5.75 to 5.79, values at which beta-elimination is still expected to occur (Figure 3.6). Moreover, these values are even higher than the pH at 0.1 MPa (5.57) where beta-elimination was observed. Consequently, the hypothesis of inhibition of the beta-elimination due to a pH drop of the sample is unlikely in MES buffer.

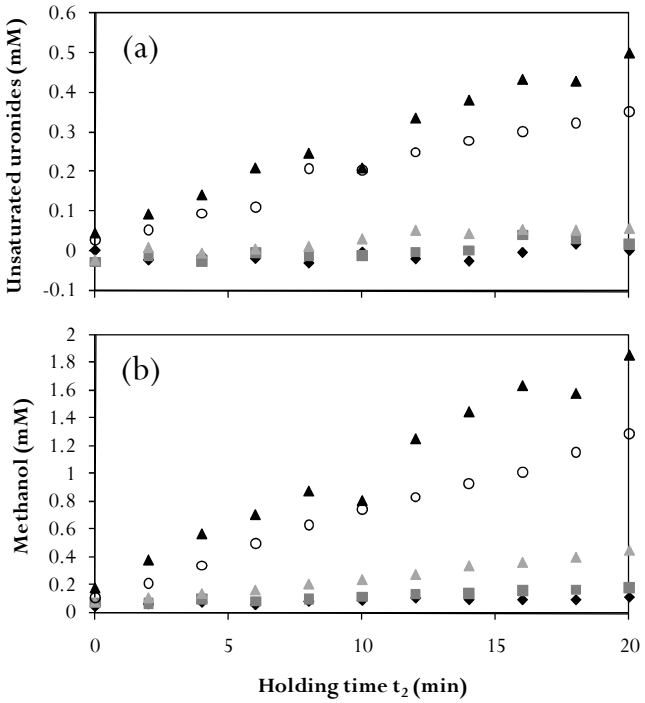


Figure 3.6: Formation of unsaturated uronides (a) and methanol (b) during treatment of pectin dissolved in 0.1 M citrate buffer at 100 °C and 0.1 MPa. ▲ pH 6.2, ○ pH 6.0, ▲ pH 5.5, ■ pH 5.0, ◆ pH 4.5.

3.3.4 Influence of DM on the rate of pectin degradation

Another possible cause for inhibition could be the concomitant and extensive pectin demethoxylation reducing the DM below a level at which beta-elimination does no longer occur. Demethoxylation and beta-elimination are two competing reactions. With increasing treatment time, the rate of beta-elimination decelerates as a result of the decreasing DM. To verify below which DM beta-elimination is significantly retarded, pectin dissolved in 0.1 M MES pH 6.5 or in 0.1 M citrate buffer pH 6.2 was treated at 100 °C and 0.1 MPa for long times. Figure 3.7 shows the formation of unsaturated uronides and changes in DM of pectin in citrate buffer. From 80 min on, the beta-elimination slowed down remarkably. This corresponded to a DM of ~ 32%. In MES buffer, very long treatment times were required to obtain a change in beta-elimination rate which was in addition less pronounced. This change was observed at a DM of ~ 40% (data not shown). One can imagine that, with further decrease of DM, the beta-elimination rate approaches zero. The samples treated for 2 min at HP/HT (Figure 3.5) had a DM of about 40%, probably not low enough to inhibit the

beta-elimination completely. However, the samples treated for longer times had DM values ranging from 26 down to 5% depending on the pressure level and treatment time. Consequently, it is plausible that the low DM caused inhibition of the beta-elimination reaction during HP/HT treatments.

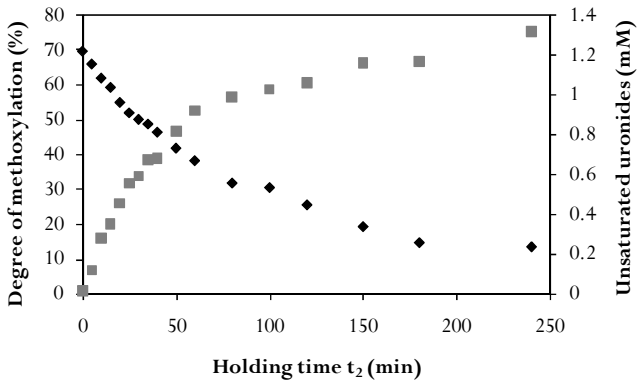


Figure 3.7: Formation of unsaturated uronides (■) and changes in DM (◆) during treatment of pectin dissolved in 0.1 M citrate buffer pH 6.2 at 100 °C and 0.1 MPa.

Considering the strong influence of pectin DM on the beta-elimination reaction rate, more complex empirical or mechanistic models taking into account the simultaneous demethoxylation reaction (instead of a simplified zero-order reaction model) may be applied to describe the kinetic data of the beta-elimination reaction. These models may allow a more quantitative description of the effect of pectin DM on the beta-elimination reaction rate. However, as the focus of this chapter was on a mechanistic understanding of the effect of HP/HT processing on the beta-elimination reaction, there was opted not to perform this elaborate quantitative analysis.

3.3.5 Importance of buffer choice: comparison of Na-phosphate and MES buffer

To verify whether buffer type and its pH dependence on temperature and pressure can have an important influence on the results, a pectin solution in 0.1 M phosphate buffer pH 6.5 was treated at 90 °C and 500, 600 and 700 MPa. The beta-elimination and demethoxylation were assayed (Figure 3.8) and compared with the results obtained at 90 °C with MES buffer pH 6.5 (data not shown). The same observations could be made: HP inhibited the beta-elimination reaction, whereas it stimulated the demethoxylation, although the latter was less pronounced. Neuman *et al.* (1973) established that the pH of a phosphate buffer shifts about -0.3 °C/100 MPa when applying HP. Under the assumption that the pH of the samples in phosphate buffer was around 4.5 to 5 during HP/HT

treatment, this low pH could account for the retarded beta-elimination (Figure 3.6). However, at this low pH a deceleration of the demethoxylation would be expected too, contrary to what was observed. Possibly, demethoxylation is stimulated by pressure to such a large extent that the inhibiting effect of the low pH is compensated.

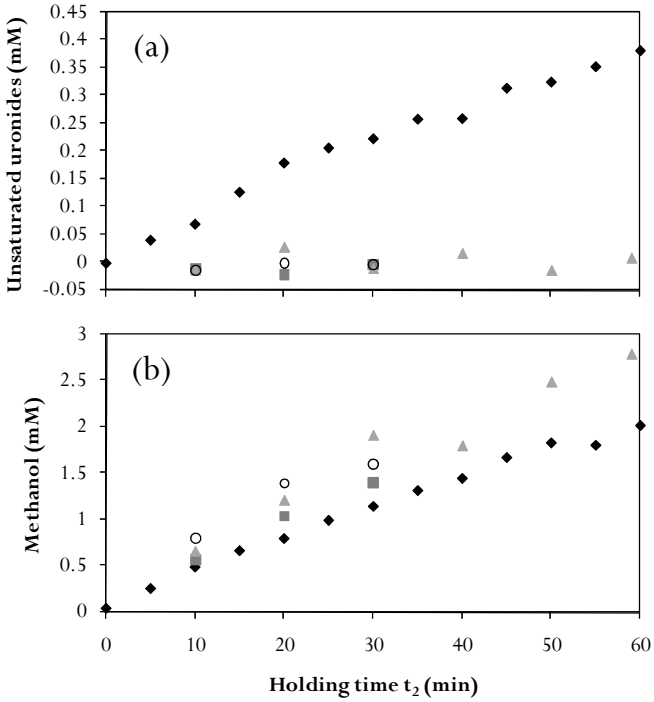


Figure 3.8: Formation of unsaturated uronides (a) and methanol (b) during treatment of pectin dissolved in 0.1 M phosphate buffer pH 6.5 at different pressure levels and 90 °C. ◆ 0.1 MPa, ■ 500 MPa, ▲ 600 MPa, ○ 700 MPa.

Although both buffers yielded the same results, different mechanisms were probably responsible for inhibition. Phosphate buffers are considered temperature-stable but pressure-labile. Therefore, the observations made could be due to a combination of a direct pressure effect and a pH shift due to the pressure. In contrast, MES buffers are considered pressure-stable and show less dependence on temperature than other pressure-stable buffers. In this buffer, large changes in pH are less likely to occur. Here, it is probably the extensive demethoxylation, resulting in pectin with a very low DM that inhibited beta-elimination, possibly in combination with a direct inhibiting pressure effect.

3.4 CONCLUSION

At 0.1 MPa and elevated temperature, beta-elimination and demethoxylation occurred at a significant rate. Irrespective of the buffer type, the rate of beta-elimination decreased when combining HT with elevated pressures, whereas the rate of demethoxylation increased. These observations are very promising for the texture of HP sterilized food products, since the beta-eliminative depolymerisation of pectin is accepted to be one of the main causes of softening of heat processed fruits and vegetables (Sila *et al.*, 2006b). Moreover, lowly methoxylated pectin was proven to enhance tissue strength by forming fortifying networks with divalent ions (e.g. Ca^{2+}) present (Sila *et al.*, 2004). The exact cause of inhibition of beta-elimination (i.e. a direct pressure effect, an indirect effect of the pressure treatment (e.g. shift in pH or DM), or a combination of both) is difficult to establish as many parameters influence the reaction rate. Concerning the choice of buffer solutions used for model systems, it has to be taken into account that the activity of hydrogen ions varies with pressure and temperature. As a consequence, the buffer pH can increase or decrease depending on the temperature, pressure, and buffer type. Generally, phosphate buffers are considered temperature-stable but pressure-labile. Hence, it may well be that the observations made in phosphate buffer during pressure treatment were not (only) due to direct pressure effects but were due to a pH shift or to a combination of both. MES buffers are considered pressure-stable and show less dependence on temperature than other pressure-stable buffers. Here, it is probably the extensive demethoxylation, resulting in pectin with a very low DM that inhibited beta-elimination. At present, buffers that are both pressure- and temperature-stable unfortunately do not exist. So, in investigating the effects of HP/HT processing on quality related food attributes, it is important to make intelligent buffer choices and to be aware of possible pH shifts, which can influence reactions taking place.

The observations made with the real food system (carrots) in the previous chapter were in line with the results obtained with pectin dissolved in MES buffer. The additional variable pressure in HP/HT processing seems responsible for an enhanced demethoxylation as compared to HT processing. Consequently, pectin with a lower DM is obtained which is less susceptible to beta-eliminative degradation. Finally, this results in a retarded texture degradation.

4 TEXTURE DEGRADATION KINETICS OF CARROTS DURING THERMAL VERSUS HIGH-PRESSURE/HIGH-TEMPERATURE PROCESSING[3]

4.1 INTRODUCTION

From the results obtained on pectin model systems (chapter 3) it could not be concluded unambiguously what causes inhibition of beta-elimination under elevated pressure and consequently whether the same inhibition occurs in real food systems. The results obtained in chapter 2 seem to confirm inhibition of beta-elimination in real food systems but, there, less intense processing conditions were applied. Therefore, in this chapter, the effect of more severe HP/HT processing conditions (approximating HP sterilization conditions) on the texture evolution of a real food system, i.e. carrots, was investigated and compared with the texture evolution of thermally processed carrots. Processing conditions were chosen assuming no synergistic effect of pressure and temperature on spore inactivation (see section 1.1.2.6). In addition, the effect of lowering the DM (by applying a HP pretreatment) and adding exogenous Ca^{2+} prior to the HP/HT treatment on the texture of carrots was investigated, since this pretreatment strategy already proved to be beneficial for the texture of thermally treated carrots (Sila *et al.*, 2004) (see section 1.2.2.4). By determining the DM of the pectin and the Ca^{2+} content of the carrots mechanistic insight was pursued.

[3] This chapter is based on the following paper:
De Roeck, A., Mols, J., Duvetter, T., Van Loey, A., Hendrickx, M. (2010). Carrot texture degradation kinetics and pectin changes during thermal versus high-pressure/high-temperature processing: A comparative study. *Food Chemistry,* 120, 1104-1112.

4.2 MATERIALS AND METHODS

4.2.1 Experimental setup to compare texture degradation kinetics of thermally and HP/HT treated carrots

Carrot samples were on the one hand thermally processed at 95, 100, 105 and 110 °C, on the other hand HP/HT processed at 95, 100 and 110 °C in combination with 600 MPa. Subsequently, the hardness of the carrots and the DM of the carrot pectin were determined. Due to the fast and extensive hardness loss at 110 °C and 0.1 MPa a relevant texture degradation rate constant could not be estimated. Therefore, 105 °C was included in the study. Figure 4.1 represents a schematic overview of the experimental setup.

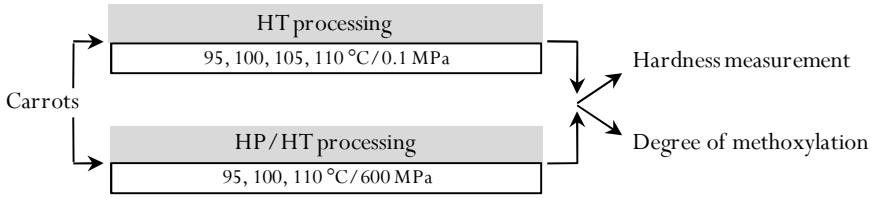


Figure 4.1: Schematic overview of the experimental setup to compare texture degradation kinetics of thermally and HP/HT treated carrots.

4.2.2 Experimental setup to investigate the effect of pretreatments on carrot texture after thermal and HP/HT processing

Carrots were on the one hand thermally (100 °C/0.1 MPa), on the other hand HP/HT (100 °C/600 MPa) processed. For both processing conditions four different sequences of pretreatment – treatment were investigated: 1) no pretreatment with the carrots processed in water (denoted by "treatment in H_2O"), 2) no pretreatment with the carrots processed in a 1.0% (w/v) calcium chloride solution ("treatment in Ca^{2+}"), 3) a HP pretreatment followed by treatment in water ("HP pretreatment"), and 4) a HP pretreatment with a subsequent Ca^{2+} soak followed by treatment in a calcium chloride solution (to prevent Ca^{2+} leaching out) ("HP pretreatment + Ca^{2+} soak"). The hardness and Ca^{2+} content of the carrots and the DM of the carrot pectin were determined. Figure 4.2 represents a schematic overview of the experimental setup. All chemicals used were of analytical grade.

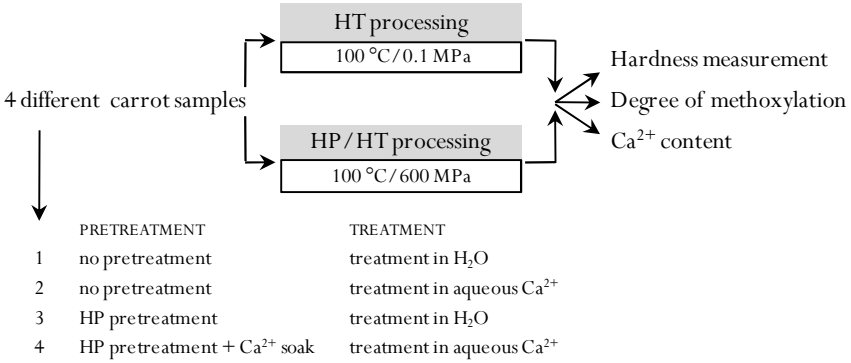


Figure 4.2: Schematic overview of the experimental setup to investigate the effect of pretreatments on carrot texture after thermal and HP/HT processing.

4.2.3 Carrots

Carrots (*Daucus carota* cultivar Nerac, 3-4 cm diameter) were obtained from a local shop in Belgium and stored at 4 °C for a maximum period of one week before use. Discs (10 mm height and 10 mm diameter) were excised from the cortex of the carrots. The cortex was chosen as it was observed to display a lower variability in hardness compared to the core.

4.2.4 Thermal treatments

Thermal treatments were executed in the same way as described in section 2.2.3. Briefly, carrot discs encapsulated in stainless steel tubes were heated in a thermostated oil bath. After a lag time of 5 min (t_1, Figure 4.3) a first sample (treatment time 0 min) was withdrawn. Subsequent samples were removed after preset holding times (t_2).

4.2.5 High-pressure/high-temperature treatments

HP/HT treatments were carried out with the same equipment and in a similar way as described in section 3.2.4. Each sample consisted of 6 carrot discs inserted in a cylindrical POM sample holder, which was filled with water and closed with a movable stopper. The sample holders were transferred to the pressure vessels already equilibrated at the final process temperature. After preheating of the samples till a certain initial temperature (t', Figure 4.3), pressure was built up very fast (10 MPa/s) (t'') which was accompanied by a temperature rise due to compression heating. After attaining the desired pressure, an equilibration time of 60 seconds (t''') was counted before the valves of the individual vessels were closed. It was observed that by increasing the equilibration time from 10 to 60 s improved isothermal-isobaric conditions were obtained. After preset holding times (t_2) the individual vessels were decompressed. Exactly 1 min after pressure release the samples were removed from the pressure vessels and cooled in an ice water bath.

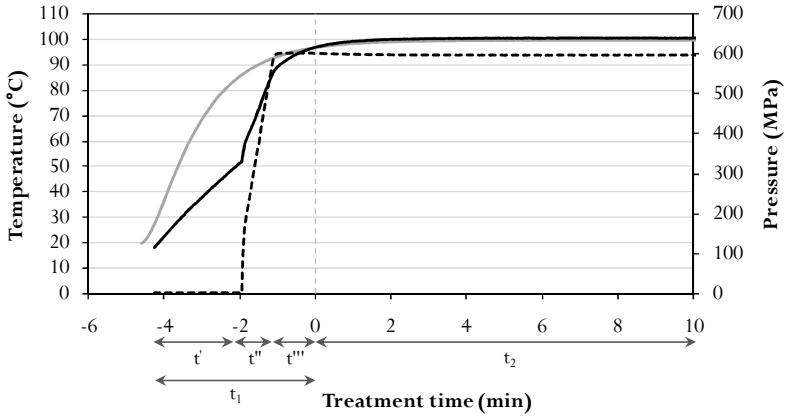


Figure 4.3: Temperature and pressure history of a carrot sample during thermal (100 °C/0.1 MPa) (grey line) versus HP/HT treatment (100 °C/600 MPa) (black full line: temperature, black dashed line: pressure). t', t'', t''' are the time of preheating, pressure build-up, and equilibration. t_1 is the total preprocess time, t_2 the holding time.

The effect of pressure build-up rate on texture degradation was also investigated. Therefore, carrot samples were HP/HT processed at 100 °C and 600 MPa applying two different pressure build-up rates. Next to the standard 600 MPa/min, carrots were also pressurized at a rate of 100 MPa/min. Hereto, pressure build-up was started at a lower initial temperature (38 °C instead of 52 °C) to reach the same final desired processing conditions (Figure 4.4).

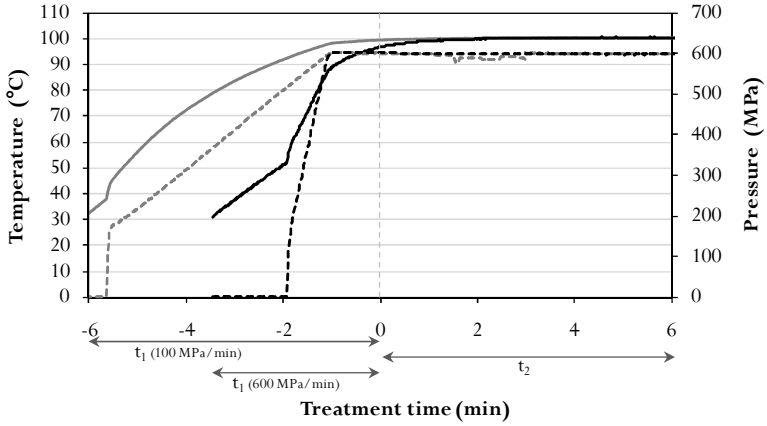


Figure 4.4: Temperature (full lines) and pressure (dashed lines) history of a carrot sample during processing at 100 °C and 600 MPa: slow (100 MPa/min) (grey lines) versus fast (600 MPa/min) (black lines) pressure build-up. t_1 and t_2 are the preprocess and holding time, respectively.

4.2.6 Pretreatments

4.2.6.1 High-pressure pretreatment

HP pretreatments were carried out in a single vessel (590 mL) HP apparatus (Engineered Pressure Systems International). This equipment allows pressures up to 600 MPa in combination with temperatures between -30 °C and 100 °C. The pressure transmitting medium was a mixture of 60% DowcalN (The Dow Chemical Company) in demineralised water. Pressurization and depressurization were almost instantaneous. Vacuum packed carrot discs were treated at 60 °C and 400 MPa for 15 min and subsequently cooled in an ice water bath. These conditions have previously been identified as optimal pretreatment conditions for texture improvement of thermally processed carrots (Sila *et al.*, 2004).

4.2.6.2 Calcium chloride soaking

Carrot discs were suspended in a 1.0% (w/v) $CaCl_2$ solution at room temperature (22 °C) and atmospheric pressure for 1 h. Afterwards, the solution was drained and the carrot discs were processed further.

4.2.7 Texture measurement

Hardness of the processed carrots was measured by a compression test as described in section 2.2.5. The mean value of the compression forces of (at least) 10 carrot cylinders was considered as a single data point.

4.2.8 Determination of pectin degree of methoxylation

The cell wall material (containing the pectin) was isolated as AIR as described in section 2.2.8. Subsequently, the DM of the carrot pectin was determined as described in section 2.2.9.

4.2.9 Determination of Ca^{2+} content of carrots

The dry ashing mineralization method as described by Vidal *et al.* (2002) was used to determine the Ca^{2+} content of the carrots. Minced carrot tissue (4.0 g) was weighed into a porcelain crucible and dried in an oven at 100 °C. The dry sample was calcinated in a muffle furnace at 600 °C. The ash was dissolved with 3.0 M HCl (10 mL) and diluted to 100 mL. The Ca^{2+} content of the obtained solution was determined (twice) using an atomic absorption spectrometer (Solaar 969) at a wavelength of 422.7 nm. A calibration curve (1 – 10 ppm) was made using a Ca^{2+} standard solution (1000 mg/L, Merck).

4.2.10 Kinetic data analysis

Most vegetables become very soft after prolonged heating at elevated temperatures, nevertheless, they retain a measurable degree of hardness. To account for this non-zero equilibrium hardness, a modified first-order reaction kinetic model, i.e. fractional-conversion, has been previously used for describing

texture degradation kinetics (Rizvi and Tong, 1997; Stoneham *et al.*, 2000; Sila *et al.*, 2004). Considering that the vegetable hardness reaches a final non-zero asymptotic value H_∞ after prolonged treatment, the rate of reduction of hardness can be expressed as follows:

$$-\frac{\partial H}{\partial t} = k(H - H_\infty)$$

where H is the hardness, t the time, and k the texture degradation rate constant (min^{-1}). Predicting the hardness at treatment time t (H_t) can be achieved by integrating previous equation:

$$H_t = H_\infty + (H_0 - H_\infty)exp(-kt)$$

The texture degradation rate constant k, the hardness at time 0 min H_0, and the hardness after prolonged treatment H_∞ were estimated by nonlinear regression analysis (SAS Statistical Software version 9.1).

The temperature dependence of the rate constants at constant pressure, expressed in terms of activation energy E_a, was estimated using the Arrhenius equation:

$$k_T = k_{ref} exp\left[\frac{E_a}{R_g}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right]$$

where E_a is the activation energy ($J \cdot mol^{-1}$), k_T the texture degradation rate constant (min^{-1}) at temperature T (K), k_{ref} the rate constant (min^{-1}) at reference temperature T_{ref} (K), and R_g the universal gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$). Likewise, E_a values were estimated by nonlinear regression analysis.

The quality of the nonlinear regression fit was expressed by the corrected correlation coefficient (Corr R^2):

$$Corr\ R^2 = \left[1 - \frac{(m-1)\left(1 - \frac{SSQ_{regression}}{SSQ_{total}}\right)}{(m-j)}\right]$$

where m is the number of observations, j the number of model parameters, and SSQ the sum of squares (Neter *et al.*, 1996).

4.3 RESULTS AND DISCUSSION

4.3.1 Comparison of texture degradation kinetics of thermally and HP/HT treated carrots

In a first phase, texture degradation kinetics of thermally and HP/HT treated carrots were compared. Hereto, carrots were treated in a temperature range

from 95 to 110 °C in combination with 0.1 MPa or 600 MPa. Long holding times were required to estimate accurate kinetic parameters.

4.3.1.1 Carrot hardness

At both pressure levels, texture degradation data could be well described by a fractional-conversion model (Figure 4.5). A summary of the estimated kinetic parameters and their respective standard errors is presented in Table 4.1. Both thermal and HP/HT treated carrots lost a considerable amount of hardness during the preprocess. This loss was dependent on the temperature and most pronounced at 0.1 MPa. Since both preprocess times hardly differed (as can be seen in Figure 4.3 for 100 °C), different preprocess times could not account for the latter. At 0.1 MPa, the carrot tissue hardness decreased very fast during subsequent holding time, reaching quickly its final residual hardness. Furthermore, the higher the temperature was, the faster the degradation. A decrease in hardness was also observed at 600 MPa. However, the degradation was around 10-fold slower. Finally, the same residual hardness was reached. Nguyen *et al.* (2007) carried out a similar experiment. They compared the effectiveness of HP/HT processing with that of conventional thermal processing in preserving carrots' texture (and other quality attributes) by matching the carrot preprocess temperature history. Similarly, they obtained a better texture preservation with HP/HT.

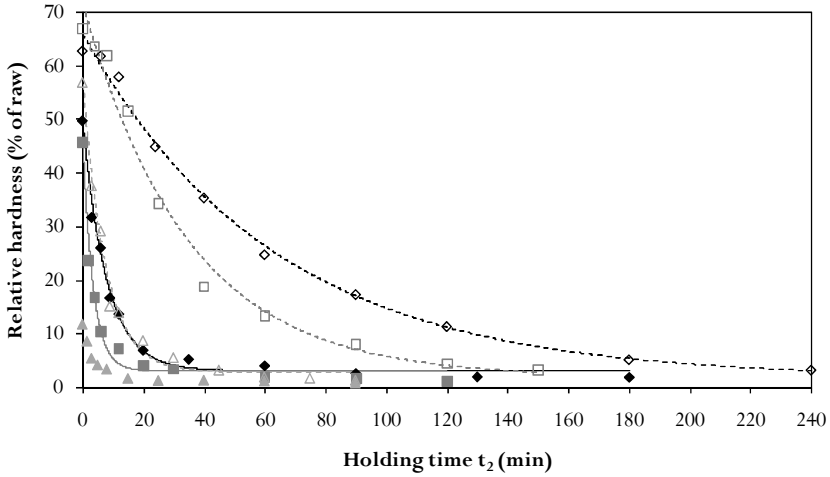


Figure 4.5: Texture degradation during thermal and HP/HT treatments of carrots, modelled using a fractional-conversion model. 0.1 MPa: ◆ 95 °C, ■ 100 °C, ▲ 110 °C. 600 MPa: ◇ 95 °C, □ 100 °C, △ 110 °C.

Table 4.1: Kinetic parameter estimates (± standard error of regression) for texture degradation of carrots, modelled by a fractional-conversion model (k: texture degradation rate constant, H_0/H_{raw}: relative hardness at time 0 min, H_∞/H_{raw}: relative hardness after prolonged treatment, E_a: activation energy).

0.1 MPa				
T (°C)	k (min^{-1})	H_0/H_{raw} (%)	H_∞/H_{raw} (%)	Corr R^2
95	0.127 ± 0.009	48.9 ± 1.4	3.0 ± 0.7	0.99
100	0.296 ± 0.030	45.0 ± 1.8	3.0 ± 0.8	0.98
105	0.530 ± 0.117	26.4 ± 2.2	2.4 ± 0.9	0.92
E_a ($kJ \cdot mol^{-1}$)	152.1 ± 15.6			0.99

600 MPa				
T (°C)	k (min^{-1})	H_0/H_{raw} (%)	H_∞/H_{raw} (%)	Corr R^2
95	0.016 ± 0.001	66.0 ± 1.4	1.7 ± 1.8	0.99
100	0.029 ± 0.004	71.4 ± 2.6	1.8 ± 2.9	0.98
110	0.134 ± 0.011	56.5 ± 1.9	2.7 ± 0.9	0.99
E_a ($kJ \cdot mol^{-1}$)	176.8 ± 7.9			0.99

So, in contrast to chapter 2 where HP/HT treatment of carrots (80 °C/600 MPa) resulted in a constant hardness during holding time, the application of HP in combination with higher temperatures was accompanied by a decrease in hardness. However, this decrease was slower than the decrease in case of thermal processing at atmospheric pressure. As mentioned previously, thermal softening of carrots is, next to membrane damage and the associated turgor pressure loss (Greve *et al.*, 1994b), mainly due to beta-eliminative degradation of the cell wall pectin resulting in an increased cell separation (Greve *et al.*, 1994a; Sila *et al.*, 2006b). The results obtained here suggest a deceleration (rather than complete inhibition) of this beta-elimination under elevated pressure. As the previous chapters have indicated that elevated pressure stimulates demethoxylation, thereby influencing the beta-elimination reaction rate, the DM of the carrot pectin was determined.

4.3.1.2 Carrot pectin degree of methoxylation

For determining the pectin DM, the treatment at 100 °C and two holding times per pressure level were selected (Table 4.2). The DM of raw carrot pectin was estimated at 63%, which is in close agreement with the value found in chapter 2 and with the values reported in literature (DM = 60 – 69%) (Siliha *et al.*, 1996; Ng and Waldron, 1997a). Very remarkable is the large difference in DM between the two pressure levels (which was also observed in chapter 2). After the preprocess (t_2 = 0 min), the pectin DM of the thermally and HP/HT treated carrots was around 62 and 48%, respectively. During 90 min holding time the

DM decreased further, again more outspoken in case of the HP/HT treated carrots. Generally, the decline can be explained by occurrence of chemical demethoxylation of pectin at HT as reported by Sajjaanantakul *et al.* (1989). The more pronounced decrease in DM in case of the HP/HT treated samples can be explained by stimulation of the chemical demethoxylation under pressure as observed in the previous chapter. However, it can not be excluded that, additionally, enzymatic demethoxylation by PME also plays a role. Jolie *et al.* (2009) have established (in model systems) that the activity of carrot PME increases from 25 °C up to around 55 °C. By further increasing the temperature, the PME activity gradually decreases due to heat inactivation of the enzyme. In Figure 4.3, it can be seen that at 0.1 MPa the carrots pass the temperature zone in which PME is activated rather quickly, whereas at 600 MPa this zone is passed more slowly due to the use of an isolating (POM) sample holder. Moreover, as already quoted in section 2.3.3.1, it has been established that HP stabilises PME (hereby retarding the heat inactivation) and even enhances its activity (Ly-Nguyen *et al.*, 2003; Jolie *et al.*, 2009). It is also possible that, due to pressurization, contact between PME and pectin was enhanced. So, as the beta-eliminative depolymerisation of pectin is considered as one of the main causes of softening of thermally treated carrots and as methyl esters are one of the main driving forces of this reaction, it is very likely that the high reduction in DM of the HP/HT treated carrots is responsible for the slower texture degradation.
Even though in chapter 2 and chapter 4 different carrot parts (respectively core and cortex) were used, similar observations could be made, namely a reduced hardness loss and a remarkably lower pectin DM in case of HP/HT processing as compared to thermal processing at 0.1 MPa. This suggests pressure is inducing the same phenomena in carrot cortex and core.

Table 4.2: Pectin DM (± standard deviation) of carrots processed at 100 °C and 0.1 MPa or 600 MPa for two different holding times t_2.

	Degree of methoxylation (%)	
	t_2: 0 min	t_2: 90 min
100 °C/0.1 MPa	61.5 ± 3.5	49.7 ± 2.7
100 °C/600 MPa	47.8 ± 1.1	31.1 ± 1.5
Raw	62.8 ± 2.3	

Although the explanation of a reduced DM retarding beta-elimination is very likely, it is not sure whether this is the sole mechanism. Biochemical systems under pressure obey the principle of Le Chatelier, which states that any phenomenon accompanied by a decrease in reaction volume is enhanced by an increase in pressure and vice versa (Yuste *et al.*, 2001). The stimulating effect of

pressure on the demethoxylation can be explained by this principle, since solvation of the charged groups created by the pectin demethoxylation is accompanied by a reduction in reaction volume. Possibly, pressure induces other reactions influencing carrot texture. The latter was also concluded from the changes in pectin solubility obtained in chapter 2.

4.3.1.3 Effect of pressure build-up rate

Apparently, the DM at the start of the holding time (t_2 = 0 min) has an important influence on the subsequent texture degradation rate. Therefore, it was investigated whether this DM could be varied by varying the pressure build-up rate, and if this would affect the further texture degradation. Hence, in addition to 600 MPa/min, carrots were pressurized at a rate of 100 MPa/min. By lowering the pressure build-up rate, preprocess time was extended with a few minutes, allowing more time for (chemical and/or enzymatic) demethoxylation to occur (Figure 4.4). However, results showed only a small difference in DM after the preprocess time. For this batch, the pectin DM of raw carrots was estimated at 65%. After fast pressure build-up the carrot pectin DM was around 51%, whereas after slow pressure build-up the DM was around 47%. This limited difference in DM had no influence on the subsequent texture degradation as similar degradation rates were observed (results not shown).

4.3.2 Effect of pretreatments on carrot texture after thermal and HP/HT processing

Lowering the susceptibility to beta-elimination (e.g. by lowering the pectin DM) is one strategy to minimize texture loss. In addition, intercellular adhesion (and consequently texture) can be enhanced by increasing the cross-links within the cell wall. Sila *et al.* (2004) have shown that the hardness of thermally processed carrot (core) discs increased according to following sequence: no pretreatment < HP pretreatment < HP pretreatment followed by a Ca^{2+} soak. The latter resulted in the hardest texture due to the occurrence of both above-mentioned effects. The HP pretreatment lowers the pectin DM by activating endogenous PME. By subsequently adding Ca^{2+}, this lowly methoxylated pectin can be cross-linked. Here, it was investigated whether the same approach can result in a similar texture improvement in case of HP/HT processing. Given the pronounced demethoxylation during a HP/HT process, the effect of carrying out the treatment in a calcium chloride solution instead of water was also investigated.

4.3.2.1 Carrot hardness

Figures 4.6 a and b show the hardness results for the thermally and HP/HT treated carrots respectively. Again, texture degradation data could be well described by a fractional-conversion model. However, due to the retarded texture degradation with certain treatments, holding times were not always long

enough to estimate H_∞ properly. At 600 MPa, it was observed that for the two treatments giving the hardest texture (i.e. treatment in Ca^{2+}, HP pretreatment + Ca^{2+} soak) the hardness was surprisingly lower at time 0 min than at time 10 min. Possibly, the minute between pressure release and sample removal from the vessel (a period during which the sample is at HT in combination with 0.1 MPa) has a certain detrimental effect on the hardness which is more pronounced at a higher residual hardness. Therefore, the experimental data at time 0 min were omitted from the kinetic modelling.

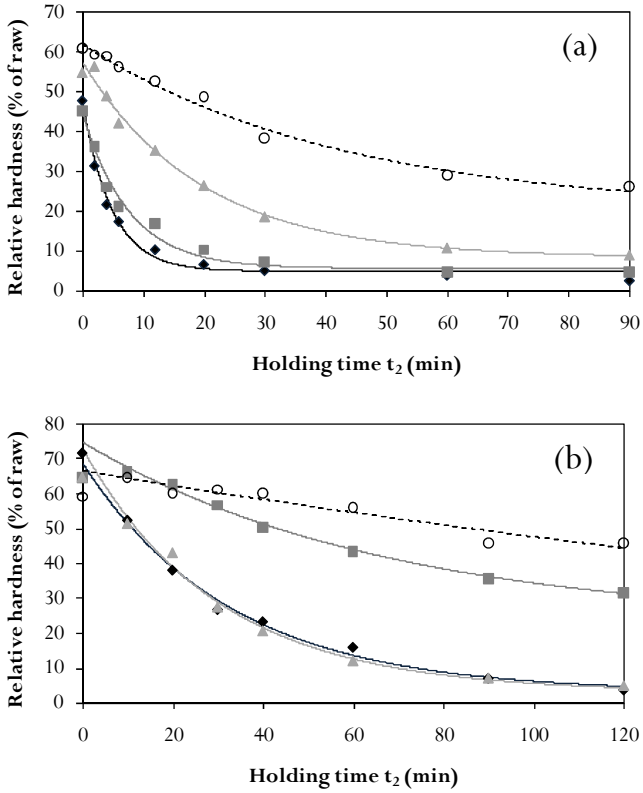


Figure 4.6: Texture degradation of pretreated carrots during a subsequent thermal (100 °C/0.1 MPa) (a) or HP/HT (100 °C/600 MPa) (b) treatment, modelled using a fractional-conversion model. ◆ treatment in H_2O, ■ treatment in Ca^{2+}, ▲ HP pretreatment, ○ HP pretreatment + Ca^{2+} soak.

Table 4.3 summarizes the estimated kinetic parameters and their respective standard errors. Firstly, the observation of slower texture degradation in case of HP/HT processing was confirmed. In addition, the results at 0.1 MPa were in accordance with the results obtained by Sila *et al.* (2004): a HP pretreatment led to a remarkable deceleration of the thermal texture degradation, even more when

followed by a Ca^{2+} soak. As in this study the carrot cortex was investigated and Sila *et al.* (2004) used the core, results again indicate that the effect of pressure is independent of carrot part. At 0.1 MPa, treating the carrots directly in a Ca^{2+} solution did not ameliorate the texture significantly. By contrast, at 600 MPa, carrying out the treatment in a Ca^{2+} solution led to a significant texture improvement, but a HP pretreatment did not. Combining a HP pretreatment with a Ca^{2+} soak also resulted in the hardest texture.

Table 4.3: Kinetic parameter estimates (± standard error of regression) for texture degradation of pretreated carrots during a subsequent thermal (100 °C/0.1 MPa) or HP/HT (100 °C/600 MPa) treatment, modelled by a fractional-conversion model (k: texture degradation rate constant, H_0/H_{raw}: relative hardness at time 0 min).

100 °C/0.1 MPa			
	k (min^{-1})	H_0/H_{raw} (%)	Corr R^2
treatment in H_2O	0.209 ± 0.019	46.8 ± 1.5	0.99
treatment in Ca^{2+}	0.131 ± 0.018	44.2 ± 1.9	0.98
HP pretreatment	0.051 ± 0.006	57.3 ± 1.5	0.99
HP pretreatment + Ca^{2+} soak	0.024 ± 0.005	61.9 ± 1.1	0.98

100 °C/600 MPa			
	k (min^{-1})	H_0/H_{raw} (%)	Corr R^2
treatment in H_2O	0.031 ± 0.004	68.7 ± 4.0	0.99
treatment in Ca^{2+}	0.015 ± 0.003	74.7 ± 1.7	0.99
HP pretreatment	0.033 ± 0.005	72.7 ± 5.2	0.98
HP pretreatment + Ca^{2+} soak	0.003 ± 0.009	66.6 ± 2.9	0.89

4.3.2.2 Carrot pectin degree of methoxylation

Table 4.4 reports the pectin DM of carrots subjected to different sequences of pretreatment and treatment. For each treatment, two holding times were selected. The DM of raw carrot pectin was estimated as 59%. The HP pretreatment lowered the DM to around 45% by stimulating endogenous PME (Sila *et al.*, 2005). At 0.1 MPa, demethoxylation was not observed during the preprocess, however, significant (chemical) demethoxylation occurred during the subsequent holding time at 100 °C. Apparently, the lower the initial DM, the smaller the subsequent decrease. This may be attributed to the lower number of esters remaining and the presence of more negative charges which results in an enhanced electrostatic repulsion between pectin and the catalysing hydroxyl ions. In contrast, at 600 MPa, marked demethoxylation occurred during the preprocess and subsequent process, observations already previously explained (section 4.3.1.2). Treating the carrots directly in a calcium chloride solution resulted in

an even more pronounced decrease. This may be ascribed to the stimulating effect of Ca^{2+} on both chemical and enzymatic demethoxylation. By binding to the carboxyl groups, electrostatic repulsion of hydroxyl ions is diminished and plant PMEs, which through their basic pI interact with negative charges, are liberated and can perform their activity (Bordenave, 1996).

4.3.2.3 Carrot Ca^{2+} content

The Ca^{2+} content of raw carrots was estimated at 0.058 mg/g (Table 4.5). Carrots which had been in contact with calcium chloride had a significantly higher Ca^{2+} content (1 – 3 mg/g carrot). Treating the carrots in a calcium chloride solution already led to a large increase. HP pretreating and subsequent soaking in a Ca^{2+} solution resulted in an even larger increase. Apparently, both the extent of tissue permeabilization and the contact time determine the Ca^{2+} uptake. For the treatment in Ca^{2+}, the difference in Ca^{2+} content at time 0 min between the two pressure levels might be due to a more intact tissue structure at 600 MPa. The larger uptake during a HP pretreatment + Ca^{2+} soak might be due to a permeable tissue in combination with a long contact time. Likewise, this can explain the increase in Ca^{2+} content after 90 min holding time. According to Rao and Lund (1986), the concentration of $CaCl_2$ (aq) in fruits and vegetables should be maximally 1.0% of the weight of the final product to prevent off flavours. So by using a 1.0% (w/v) $CaCl_2$ solution, the Ca^{2+} concentration of the carrots is safely below this limit.

Table 4.4: Pectin DM (± standard deviation) of carrot samples subjected to various sequences of pretreatment and treatment (at 100 °C and 0.1 MPa or 600 MPa for two different holding times t_2).

	Degree of methoxylation (%)				
	Raw or after pretreatment	**100 °C/0.1 MPa**		**100 °C/600 MPa**	
		t_2: 0 min	t_2: 90 min	t_2: 0 min	t_2: 90 min
treatment in H_2O	58.8 ± 1.8	59.7 ± 1.6	47.0 ± 1.2	50.0 ± 1.1	28.9 ± 0.6
treatment in Ca^{2+}	58.8 ± 1.8	60.1 ± 1.1	46.2 ± 1.1	43.0 ± 0.6	27.7 ± 0.4
HP pretreatment	45.7 ± 1.0	46.5 ± 0.9	39.3 ± 0.9	39.6 ± 1.1	25.7 ± 0.6
HP pretreatment + Ca^{2+} soak	44.8 ± 1.3	42.9 ± 0.7	39.2 ± 0.6	36.4 ± 0.9	27.5 ± 0.6

Table 4.5: Ca^{2+} content (± standard deviation) of carrot samples subjected to various sequences of pretreatment and treatment (at 100 °C and 0.1 MPa or 600 MPa for two different holding times t_2).

	Ca^{2+} content (mg Ca^{2+}/g carrot)				
	Raw or after pretreatment	**100 °C/0.1 MPa**		**100 °C/600 MPa**	
		t_2: 0 min	t_2: 90 min	t_2: 0 min	t_2: 90 min
treatment in H_2O	0.058 ± 0.002	0.074 ± 0.001	0.081 ± 0.000	0.112 ± 0.001	0.083 ± 0.001
treatment in Ca^{2+}	0.058 ± 0.002	1.338 ± 0.126	1.840 ± 0.171	0.882 ± 0.014	1.989 ± 0.060
HP pretreatment	0.074 ± 0.002	0.110 ± 0.000	0.117 ± 0.000	0.103 ± 0.000	0.109 ± 0.000
HP pretreatment + Ca^{2+} soak	2.745 ± 0.003	2.614 ± 0.021	2.809 ± 0.086	2.476 ± 0.076	2.877 ± 0.144

4.3.2.4 Correlation of hardness with pectin DM and Ca^{2+} content carrots

In the context of rheological characterization of Ca^{2+} mediated gels, the influence of Ca^{2+} concentration on the gelation of lowly methoxylated pectin is mostly described in terms of a stoichiometric ratio $R = 2[Ca^{2+}]/[COO^-]$ which relates the Ca^{2+} concentration to the amount of non-methoxylated GalA residues (Garnier *et al.*, 1993; Capel *et al.*, 2006). Theoretically, a ratio of 0.5 implies that all Ca^{2+} is bound to pectin in so-called egg-boxes. Further addition of Ca^{2+} results in other, less specific, electrostatic pectin-calcium interactions which contribute minimally to gel strength. This R ratio was calculated for some of the samples in this study to assess the extent of possible cross-linking in the carrot cell wall. Table 4.6 shows the R ratio for the differently treated carrots at the start of the holding time. The COO^- concentration could be calculated from the GalA content of the AIR and the DM of the carrot pectin, under the assumption that at the pH of carrots (which is around 6) all non-methoxylated GalA residues are dissociated.

Changing the processing medium from water to a calcium chloride solution had a slight influence on the hardness when treating carrots at 0.1 MPa (Figure 4.6 a). As the carrots only differed in Ca^{2+} content and not in DM, enhanced cross-linking of non-methoxylated GalA residues present could be responsible. Carrots treated in water had an R value equal to 0.17, whereas carrots treated in a Ca^{2+} solution had an R value of 3.44. The former implies that, even under the assumption that all Ca^{2+} present in the carrot tissue is available for binding to pectin, there will still remain free non-methoxylated GalA residues. In the latter case, the high R value indicates that sufficient Ca^{2+} is present to cross-link all non-methoxylated residues, hereby fortifying the tissue. A HP pretreatment had a remarkable influence on the hardness of thermally treated carrots. Because of a low DM, the carrot pectin was less susceptible to beta-eliminative depolymerisation. The additional effect on hardness of a Ca^{2+} soak after a HP pretreatment can be explained as previously. After the HP pretreatment (R value of 0.21), there are many free non-methoxylated GalA residues left. After the HP pretreatment + Ca^{2+} soak enough Ca^{2+} is present (R = 4.44) to theoretically bind all free GalA residues, hereby improving the cell adhesion.

At 600 MPa, different observations were made (Figure 4.6 b). A HP pretreatment had no influence on the subsequent texture degradation. When comparing the texture degradation rate constants of the thermal treatment preceded by a HP pretreatment (0.051 min^{-1}) and the HP/HT treatment with (0.033 min^{-1}) or without (0.031 min^{-1}) a HP pretreatment, it can be noticed that all constants are of the same order of magnitude. Apparently, seen the comparably low DM at time 0 min, the HP/HT preprocess can be regarded as a HP pretreatment implicitly present. This can explain why a separate HP

pretreatment has no influence on the texture degradation of HP/HT treated carrots. Analogously, the rate constants of the thermal treatment preceded by a HP pretreatment + Ca^{2+} soak (0.024 min^{-1}) and the HP/HT treatment carried out in a Ca^{2+} solution (0.015 min^{-1}) were comparable. As the pectin DM at the start of the holding time was identical but the carrot Ca^{2+} content differed, this resulted in a different R value (4.44 and 1.38 respectively). As both values were higher than 0.5 and seen the similar texture degradation, it can be concluded that there was an excess of Ca^{2+}. A HP pretreatment followed by a Ca^{2+} soak resulted in the highest texture improvement, texture degradation being really limited.

Table 4.6: Stoichiometric ratio R = 2[Ca^{2+}]/[COO^-] correlating Ca^{2+} concentration to the amount of non-methoxylated GalA residues in carrots treated at 100 °C and 0.1 MPa or 600 MPa for 0 min holding time t_2.

	Stoichiometric ratio R = 2[Ca^{2+}]/[COO^-]	
	100 °C/0.1 MPa	**100 °C/600 MPa**
	t_2: 0 min	t_2: 0 min
treatment in H_2O	0.17	0.23
treatment in Ca^{2+}	3.44	1.38
HP pretreatment	0.21	0.17
HP pretreatment + Ca^{2+} soak	4.44	3.12

The observed differences in hardness evolution could be explained based on the Ca^{2+} content of the carrots and the DM of the carrot pectin. However, two remarks have to be made. Firstly, concerning the DM, only the quantity of non-methoxylated GalA residues was taken into account. However, also the distribution of non-methoxylated GalA residues can be of importance (Goldberg *et al.*, 1996). Depending on whether chemical or enzymatic demethoxylation dominates, this distribution can vary from random to more blockwise, hereby influencing pectin's functionality. For example, a HP pretreatment is expected to result in a blockwise pattern of demethoxylation as endogenous carrot PME is mainly stimulated. To date, the pattern after a HP/HT preprocess is not known as it has not yet been elucidated which kind of demethoxylation is predominant. Secondly, it can not be excluded that other mechanisms also play a role.

4.4 CONCLUSION

The potential of HP sterilization, as an alternative for thermal sterilization, regarding texture preservation of processed fruits and vegetables was investigated on a kinetic basis. By combining a thermal treatment with elevated pressure, the

thermal texture degradation of carrots was remarkably slowed down. Extensive pectin demethoxylation during the HP/HT preprocess could be responsible for a retarded beta-elimination, which is considered as the main cause of thermal softening of carrots. The formerly established pretreatment strategy of lowering the DM and adding exogenous Ca^{2+} for texture improvement of thermally processed fruits and vegetables was also successful in case of HP/HT processing. However, an almost similar outcome was obtained by HP/HT treating the carrots directly in a calcium chloride solution without applying a pretreatment. Given the strong demethoxylation, the HP/HT dynamic build-up phase can be regarded as a HP pretreatment implicitly present. Obviously, excluding a separate pretreatment step will lead to time savings and a lower cost.

The results obtained with carrots were in accordance with the results obtained with pectin dissolved in MES buffer (chapter 3) where extensive pectin demethoxylation was probably responsible for an inhibited beta-elimination. In contrast to chapter 2 where HP treatment at 80 °C resulted in a constant hardness of carrots during holding time, the hardness of HP treated carrots at higher temperatures (95 – 110 °C) did actually decrease during holding time. This different behaviour could be due to the lower DM at the start of the holding time (possibly due to the longer preprocess time) and/or to the lower processing temperature in the former case. In chapter 3, it was observed (at 0.1 MPa) that the beta-elimination rate constant shows a rather strong dependence on temperature. Consequently, it is possible that with increasing temperature beta-elimination is much more pronounced that lowering the DM has relatively less influence on the reaction rate.

5 TEXTURE PRESERVATION OF PROCESSED CARROTS: COMPARISON OF EQUIVALENT THERMAL AND HIGH-PRESSURE PROCESSES

5.1 INTRODUCTION

Processing conditions used in the previous chapters do not truly reflect p-T-t combinations applied in industry. Treatment times, for instance, were always taken much longer than industrially applied to enable a better investigation of underlying mechanisms and a more accurate estimation of kinetic parameters. This last chapter is more practically oriented. The aim was, by applying industrially relevant processing conditions (both pasteurization and sterilization intensities), to compare the effect of equivalent thermal and HP processes on hardness and microstructure of carrots. Equivalent processes are defined as processes resulting in the same degree of microbial inactivation. As sterilization is always accompanied with a certain degree of texture degradation, approaches to reduce this texture loss were also studied. In case of thermal sterilization, the well-established pretreatment strategy of preheating at mild temperature followed by soaking in a Ca^{2+} solution was applied (see section 1.2.2.4). In case of HP sterilization, carrots were either processed in water, either in a Ca^{2+} solution, as it was observed in chapter 4 that by doing so carrot texture was significantly enhanced.

5.2 MATERIALS AND METHODS

5.2.1 Experimental setup

A schematic overview of the experimental setup is presented in Figure 5.1. Carrot discs were on the one hand subjected to equivalent thermal and HP pasteurization processes, on the other hand equivalent thermal and HP sterilization processes. The pasteurization process was designed in order to obtain a 6 log reduction of non-proteolytic *C. botulinum* spores. In case of sterilization, the aim was a 12 log reduction of proteolytic *C. botulinum* spores. Afterwards, processed carrots were stored at 4 °C for several days. On different days, hardness and microstructure of the carrots were analysed. In addition, in case of thermal sterilization, carrots were preheated at mild temperature and soaked in a Ca^{2+} solution. HP sterilized carrots were either processed in water, either in a Ca^{2+} solution. All chemicals used were of analytical grade.

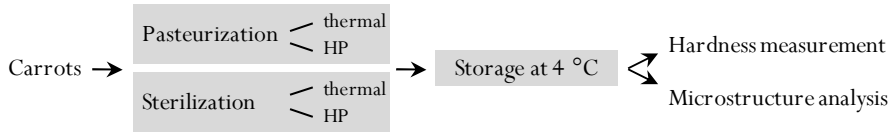


Figure 5.1: Schematic overview of the experimental setup.

5.2.2 Carrots

For the pasteurization experiment, carrots (*Daucus carota* cultivar Nairobi, 3 cm diameter) were obtained from a local grower in Norwich (UK). Carrots for the sterilization experiment (*Daucus carota* cultivar Nerac, 3-4 cm diameter) were bought at a local shop in Belgium. Carrots were stored at 4 °C for a maximum period of one week before use. Discs (10 mm height and 10 mm diameter) were excised from the cortex of the carrots.

5.2.3 Thermal pasteurization

Carrot discs (10) encapsulated in stainless steel tubes (110 mm long, 13 mm internal diameter, and 1 mm thickness) filled with demineralised water were heated in a thermostated water bath at 90 °C to obtain a process value $^{10\,°C}P_{90\,°C}$ of 10 min at the centre of the carrot discs. The temperature profile in the carrot discs was recorded using type T thermocouples connected to a thermocouple box (TR9216, Ellab) and a CMC-92 data acquisition system (Ellab). Pasteurized samples were cooled in an ice water bath and stored at 4 °C up to 7 days.

5.2.4 High-pressure pasteurization

Samples were HP pasteurized in the single vessel HP apparatus described in section 4.2.6.1. Carrot discs were packed in a polyethylene bag filled with

demineralised water and treated at 45 °C (initial temperature) and 600 MPa for 20 min. Processed samples were cooled in an ice water bath and stored at 4 °C.

5.2.5 In-pack thermal sterilization

Glass jars (370 mL volume, 99 mm height, 80 mm diameter) were filled with carrot discs (raw or pretreated (see section 5.2.6)) and uncalibrated carrot pieces to a fill weight of 190 ± 0.5 g. Demineralised water or a 1.0% (w/v) $CaCl_2$ solution was added as a brine, leaving a headspace of 5 mm. Samples were sterilized using a static steriflow pilot retort (Barriquand) at 117 °C to obtain a process value $^{10\ °C}F_{121.1\ °C}$ of 3 min at the coldest point of the glass jars. Temperature profiles of retort and samples were recorded as described in section 5.2.3. Sterilized samples were stored at 4 °C up to 9 days.

5.2.6 Pretreatment in case of thermal sterilization

Vacuum packed carrot discs were heated at 60 °C for 40 min in a temperature controlled water bath, cooled in an ice water bath, and subsequently soaked for 1 h at room temperature (22 °C) and atmospheric pressure in a 1.0% (w/v) $CaCl_2$ solution. These conditions have previously been identified as optimal pretreatment conditions for texture improvement of thermally processed carrots (Smout *et al.*, 2005).

5.2.7 High-pressure sterilization

HP/HT treatments were carried out with the same equipment and in a similar way as described in section 4.2.5. Each sample consisted of 6 carrot discs inserted in a cylindrical POM sample holder, which was filled with water or a 1.0% (w/v) $CaCl_2$ solution. Pressure vessels were equilibrated at 117 °C. After preheating of the samples and pressure build-up (till 600 MPa), an equilibration time of 60 seconds was counted before the valves of the individual vessels were closed. After a certain holding time (to obtain a process value $^{10\ °C}F_{121.1\ °C}$ of 3 min) vessels were decompressed.

As the amount of carrots discs that could be treated in one pressure cycle was limited, 3 cycles were necessary to produce enough material for the different analyses. Figure 5.2 shows the T-t profile of 3 different runs. Good reproducibility can be observed. For each pressure cycle the necessary holding time to reach an $^{10\ °C}F_{121.1\ °C}$ of 3 min was determined during the course of the process based on the temperature reached after isolation of the vessels. Exactly 1 min after pressure release the samples were removed from the pressure vessels and cooled in an ice water bath. Processed samples were stored at 4 °C.

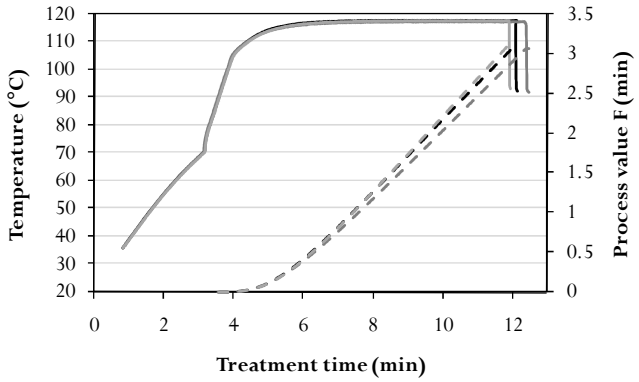


Figure 5.2: Temperature evolution of a carrot sample (full lines) during 3 different pressure cycles (at 600 MPa) and the corresponding process value $^{10\ °C}F_{121.1\ °C}$ (dashed lines).

5.2.8 Texture measurement

Hardness of the processed carrots was measured by a compression test as described in section 2.2.5. The mean value of the compression forces of (at least) 20 carrot discs was considered as a single data point.

5.2.9 Microstructure analysis

Carrot samples were prepared and their microstructure analysed as described in section 2.2.6.

5.3 RESULTS AND DISCUSSION

5.3.1 Thermal versus high-pressure pasteurization

5.3.1.1 Processing conditions

The thermal pasteurization process was designed in order to obtain a 6 log reduction of non-proteolytic *C. botulinum* spores which corresponds to a process value $^{10\ °C}P_{90\ °C}$ of 10 min (Peck, 2006; Silva and Gibbs, 2010). Thermally treated carrots reached the required process value after a treatment time of 12 min in a water bath at 90 °C (Figure 5.3). By extrapolation of available literature data (Reddy *et al.*, 1999), a HP process of 20 min at 45 °C and 600 MPa was assumed to result in a 6 log reduction of non-proteolytic *C. botulinum* spores.

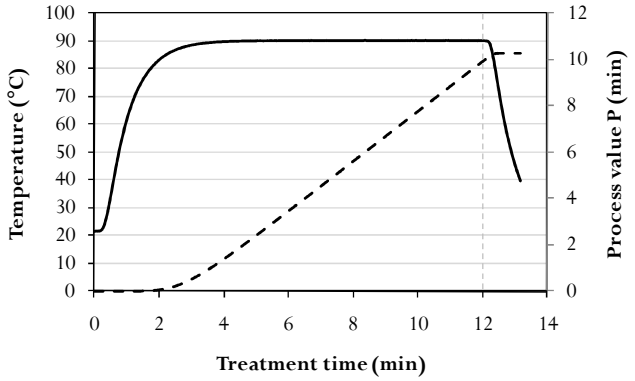


Figure 5.3: Temperature evolution of a carrot sample (full line) placed in a water bath at 90 °C for 12 min and the corresponding process value $^{10\,°C}P_{90\,°C}$ (dashed line).

5.3.1.2 Carrot hardness and its evolution upon storage

Figure 5.4 shows the residual hardness of carrot discs after equivalent thermal and HP pasteurization processes. Thermal pasteurization resulted in 80% hardness loss. By contrast, HP pasteurized carrots only lost 40% of their hardness. Upon storage at 4 °C during 7 days, no changes in hardness were observed. As mentioned previously, thermal softening of carrots is, next to membrane damage and the associated turgor pressure loss (Greve *et al.*, 1994b), mainly due to beta-eliminative depolymerisation of pectin (Greve *et al.*, 1994a; Sila *et al.*, 2006b). In case of the HP process, texture loss was probably only due to membrane damage and turgor pressure loss (resulting from the instantaneous pulse action of the pressure) (Basak and Ramaswamy, 1998) as the temperature was not high enough to induce beta-elimination.

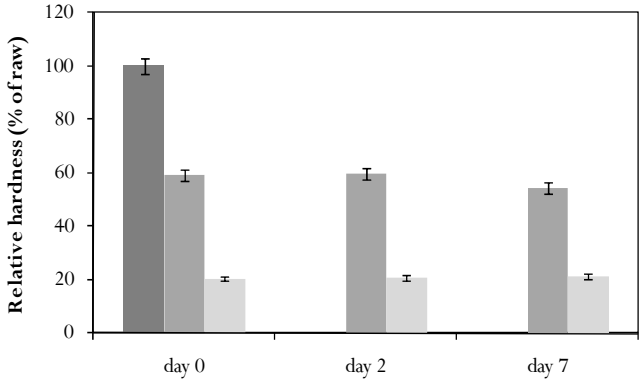


Figure 5.4: Hardness (± standard error) of carrot discs after a pasteurization process aimed at a 6 log reduction of non-proteolytic *C. botulinum* spores and its evolution upon storage at 4 °C. ■ raw, ■ HP pasteurization, ■ thermal pasteurization.

5.3.1.3 Carrot microstructure

Raw, HP pasteurized, and thermally pasteurized carrot tissue clearly represent different characteristics (Figure 5.5). Raw carrot tissue shows well-defined, well-stained cell walls. HP pasteurized carrot tissue also displays well-defined cell walls but cells are more irregular in shape, probably due to the applied pressure distorting the tissue (Araya *et al.*, 2007). Thermally pasteurized carrot tissue has more weakly coloured cell walls showing some cell wall separation with in between purple coloured voids. These observations are probably due to heat induced solubilisation of the intercellular cementing pectin facilitating cell wall loosening (Waldron *et al.*, 2003). The purple colour of the voids might be due to accumulation of dissolved pectin. No changes in microstructure were observed during storage at 4 °C (results not shown).

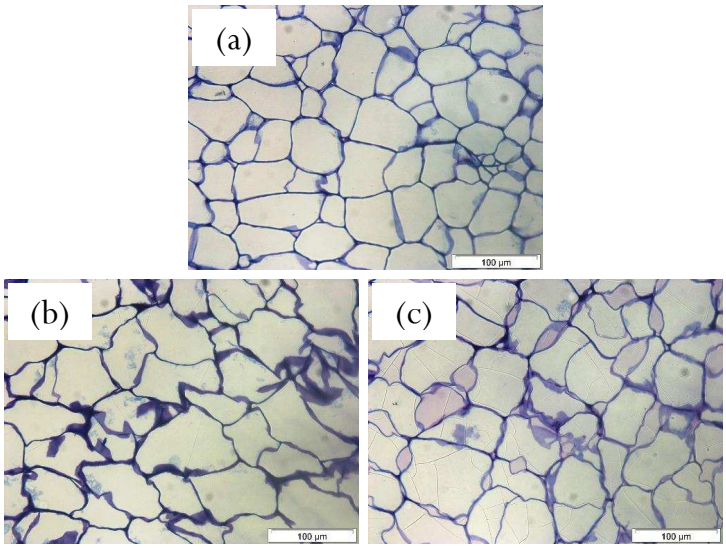


Figure 5.5: Micrographs of (a) raw, (b) HP pasteurized, and (c) thermally pasteurized carrot tissue. The pasteurization process aimed at a 6 log reduction of non-proteolytic *C. botulinum* spores.

The macroscopic and microscopic observations corresponded very well. HP pasteurized carrots showed limited hardness loss. Likely, this loss was only due membrane damage and turgor pressure loss as it appeared from the good cell adhesion in the micrographs that beta-elimination had not occurred. In contrast, thermally pasteurized carrots showed more pronounced texture loss. This was probably due to the combined effect of turgor pressure loss and heat induced degradation of pectin as cell adhesion seemed diminished.
The observations are also in accordance with the results obtained in chapter 2. Carrot tissue treated at HT (80 or 100 °C) and 0.1 MPa showed a pronounced

texture loss (Figure 2.2) and cell wall separation (Figure 2.3). In contrast, carrots treated at 80 °C and 600 MPa only lost 40% of their initial hardness and no (or limited) cell wall separation was observed. The results obtained here seem to confirm that at 80 °C and 600 MPa texture loss was mainly due to turgor pressure loss and hardly or not to beta-elimination.

5.3.2 Thermal versus high-pressure sterilization

5.3.2.1 Processing conditions

Thermal and HP sterilization processes were designed in order to obtain a 12 log reduction of proteolytic *C. botulinum* spores which corresponds to a process value $^{10\ °C}F_{121.1\ °C}$ of 3 min (Ganzle *et al.*, 2007; Leadley *et al.*, 2008). It was assumed that pressure had no effect on microbial spore inactivation. Figure 5.6 shows the temperature evolution (starting from 40 °C) of a carrot sample during both processes. Three different phases could be distinguished in the T-t profile of the retort: a coming-up phase of 9 min, a holding phase at 117 °C of 17 min and a cooling phase. Because of heat transfer limitations, the T-t profile inside a carrot sample differed from that of the retort; samples never reached the actual process temperature of 117 °C. The HP process consisted of a preheating phase, a pressure build-up phase and a holding phase. Due to adiabatic decompression cooling was almost instantaneously. HP sterilized samples reached 117 °C in a rather short time.

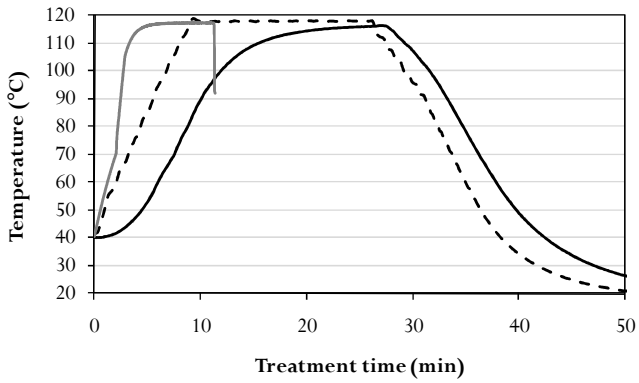


Figure 5.6: Temperature evolution of retort (dashed line), thermally sterilized carrot sample (black line), and HP sterilized carrot sample (grey line); both processes resulting in a process value $^{10\ °C}F_{121.1\ °C}$ of 3 min.

It can be noted that, due to the different dimensions of a POM sample holder and a glass jar, comparison of both processes is rather precarious. However, due to the uniform and instantaneous pressure transmission and compression heating the dimensions of the HP container are not expected to play a role. The only risk when using larger containers is that they require longer preheating times which

could negatively influence texture. However, preheating temperatures are mostly below temperatures at which pectin degradation occurs.

5.3.2.2 Carrot hardness and its evolution upon storage

Figure 5.7 shows the residual hardness of carrot discs after equivalent thermal and HP sterilization processes. Thermal sterilization resulted in extensive tissue softening: only 2% of the initial hardness remained. By contrast, HP sterilized carrots retained 12% of their initial hardness. Although (slightly) higher temperatures were reached in case of HP sterilization, the hardness loss was less. This could be attributed to faster heating and cooling, and to the consequently shorter treatment time (Figure 5.6). However, the occurrence of other reactions or differences in rates of reactions occurring could also be responsible. In the previous chapters, it was observed that during the first phases of a HP/HT process (preheating and pressure build-up) extensive pectin demethoxylation (chemical and/or enzymatic) occurs. The obtained lowly methoxylated pectin is less susceptible to beta-eliminative degradation and can form fortifying cross-links with endogenous Ca^{2+}. Although at 0.1 MPa there was more time for demethoxylation to occur (seen the slower heating rate), pronounced demethoxylation was less likely as the additional variable HP, responsible for the enhanced demethoxylation, was not present. Upon storage at 4 °C, no changes in hardness were observed. Storage could not be performed at room temperature because aseptic handling of processed carrots was not possible.

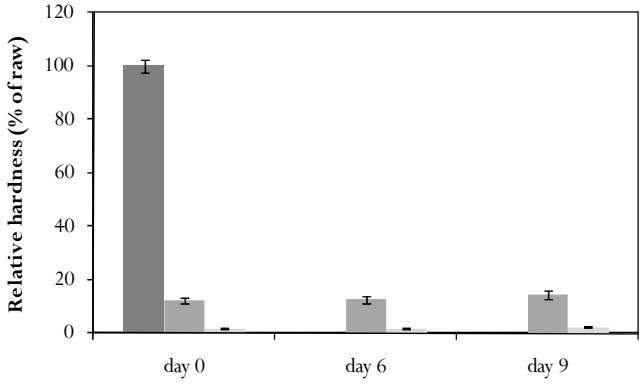


Figure 5.7: Hardness (± standard error) of sterilized carrot discs (process value $^{10\,°C}F_{121.1\,°C}$ of 3 min) and its evolution upon storage at 4 °C. ■ raw, ■ HP sterilization, ■ thermal sterilization.

5.3.2.3 Carrot microstructure

Figure 5.8 shows micrographs (taken at different magnifications) of raw, HP sterilized and thermally sterilized carrot tissue. The three tissues clearly show

different characteristics. Raw carrot tissue has intensely blue coloured, well-defined cell walls. HP sterilized carrot tissue has rather intensely purple coloured cell walls which are not, partly or fully separated from each other. In the latter two cases, pink coloured voids appear between the separated cell walls. Thermally sterilized carrot tissue also has purple, but much more weakly coloured cell walls. Cell wall separation is also visible, however less clearly as the voids have no pink colour. No changes in microstructure were observed during storage at 4 °C (results not shown).

How can these differences be explained? In raw carrot tissue, intact pectin cross-links neighbouring cell walls, resulting in a strong intercellular adhesion. However, at HT, pectin undergoes beta-eliminative depolymerisation. This degradation leads to pectin solubilisation and, consequently, to decreased cell adhesion, resulting in tissue softening. The observations made in the HP sterilized tissue are probably due to this heat induced solubilisation of the intercellular pectin. The pink colour of the voids might be due to accumulation of dissolved pectin. The weak colour of the thermally sterilized tissue suggests that the tissue is already in a further stage of degradation. Probably, the pectin was also prone to beta-eliminative degradation but much more extensively due to the longer process time and possibly higher DM of the carrot pectin. The pectin might have been intensively degraded, becoming very soluble, and might have leached out to the brine (instead of accumulating between cell walls). In chapter 2 (Figure 2.6), a high amount of solubilised cell wall polymers was found in the brine of thermally processed carrots but not in the brine of HP/HT processed carrots, confirming above-mentioned hypothesis. So, the microscopic observations suggest different degrees of pectin solubilisation in HP and thermally sterilized carrot tissue. This could explain the larger texture loss in case of thermal sterilization.

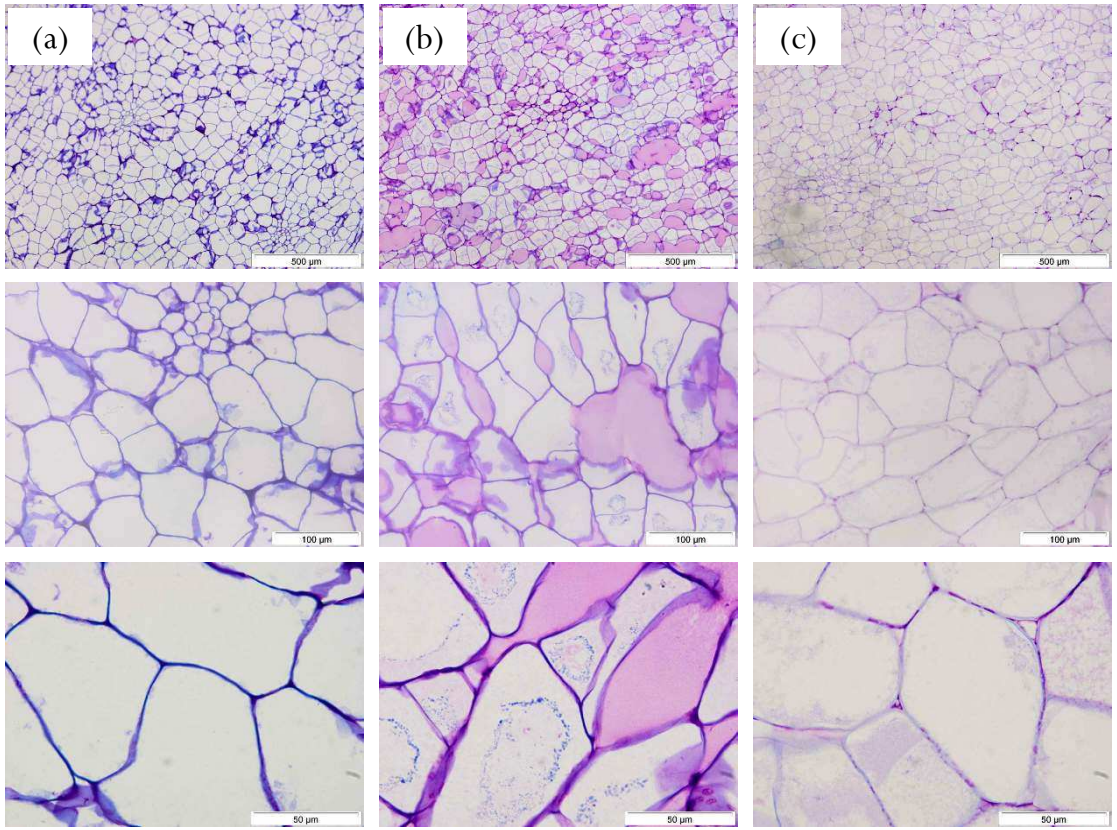


Figure 5.8: Micrographs (taken at different magnifications) of (a) raw, (b) HP sterilized, and (c) thermally sterilized carrot tissue.

5.3.2.4 Texture improvement of sterilized carrots

As both thermally and HP sterilized carrots lost a considerable amount of hardness during processing, it was tried to diminish this loss (Figure 5.9). By replacing the treatment medium water with a Ca^{2+} solution, hardness of HP sterilized carrots was increased by a factor 3 compared to HP sterilized carrots treated in water. In case of a thermal sterilization, the well-established pretreatment strategy of preheating at mild temperature followed by soaking in a Ca^{2+} solution confirmed to be successful. The residual hardness of the carrots was 14% which is a reduction of the texture loss by a factor 6 to 7 compared to non-pretreated thermally sterilized carrots. However, in the latter case, residual hardness only equalled the hardness of HP sterilized carrots treated in water, demonstrating that, concerning texture preservation of carrots, HP sterilization has much more potential.

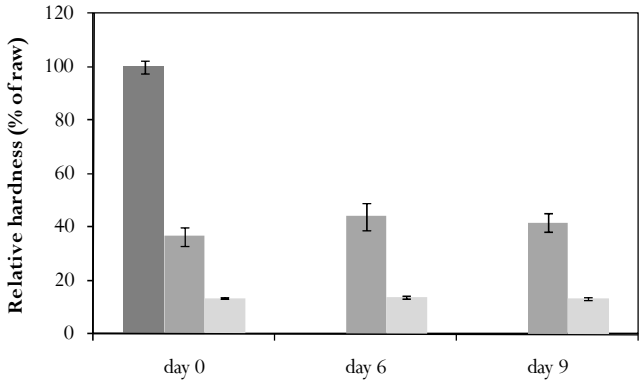


Figure 5.9: Hardness (± standard error) of sterilized carrot discs (process value $^{10\,°C}F_{121.1\,°C}$ of 3 min) and its evolution upon storage at 4 °C. ■ raw carrots, ■ carrots HP sterilized in a Ca^{2+} solution, ■ pretreated carrots thermally sterilized.

Figure 5.10 shows micrographs of HP and thermally sterilized carrot tissues. Same observations as previously can be made. HP sterilized carrot tissue displays intensely coloured cell walls which are not, partly or fully separated from each other and pink coloured voids. Thermally sterilized carrot tissue has much more weakly coloured cell walls and voids that are not coloured. When comparing Figure 5.10 with Figure 5.8, HP sterilized carrot tissue treated in a Ca^{2+} solution seems to show less cell separation and smaller voids. This could be due to enhanced Ca^{2+} cross-linking of pectin resulting in a better cell adhesion. Differences between the pretreated and non-pretreated thermally sterilized tissues are less clear. However, cell walls in case of pretreated carrots look darker coloured, suggesting enhanced cell adhesion.

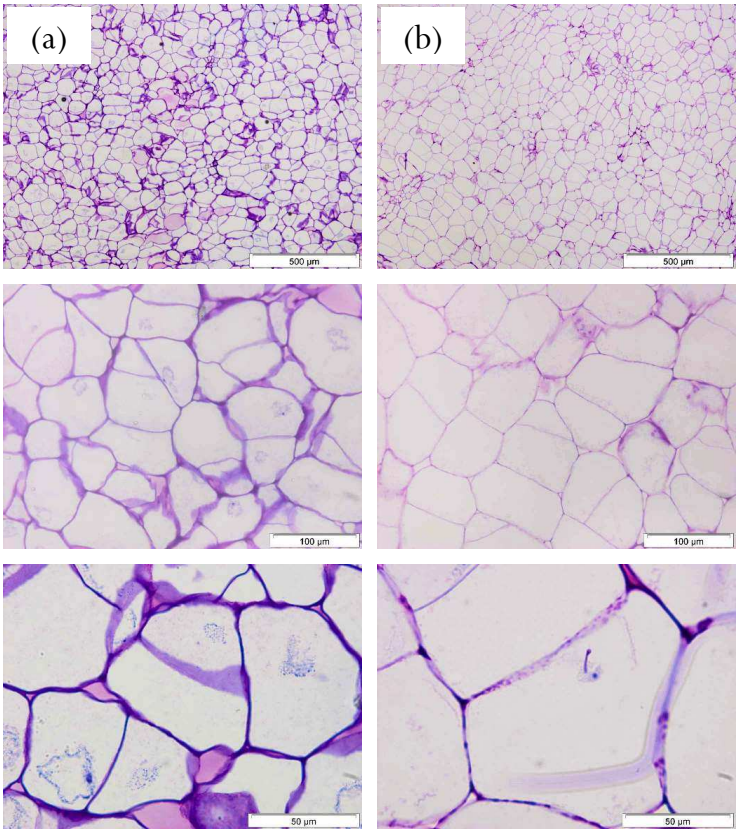


Figure 5.10: Micrographs (taken at different magnifications) of (a) carrots HP sterilized in a Ca^{2+} solution, (b) pretreated carrots thermally sterilized (process value $^{10\,°C}F_{121.1\,°C}$ of 3 min).

5.4 CONCLUSION

Concerning texture preservation of processed carrots, HPP proved to be preferred to thermal processing, both for pasteurization and sterilization intensities. In case of HP pasteurization, the limited texture loss was expected as processing temperatures were not high enough to support beta-eliminative pectin degradation. Both thermal and HP sterilization processes were accompanied by significant hardness loss, however less pronounced in case of HP sterilization. The latter could be attributed to two effects: (i) faster heating and cooling rates and correspondingly shorter treatment times and (ii) enhanced pectin demethoxylation during combined HP/HT processing resulting in lowly

methoxylated pectin which is less susceptible to beta-eliminative depolymerisation and can form fortifying networks with Ca^{2+} present.
Based on the current knowledge, no synergistic effect of pressure and temperature on microbial spore inactivation must be assumed. In case of a synergistic effect, next to shorter treatment times, lower maximum temperatures can be applied, expecting to result in an even better preservation of quality characteristics susceptible to heat (e.g. texture).

GENERAL CONCLUSIONS

The objective of this work was to investigate the potential of combined HP/HT processing in preserving texture of processed fruits and vegetables. Adding elevated pressure to a thermal process might influence texture in several ways. Instantaneous and volumetric heating during pressurization and cooling during depressurization results in reduced processing times. Next to this, the pressure by itself might influence texture by affecting texture related phenomena.

Thermal processing at 0.1 MPa caused extensive softening of carrot tissue. Hardness decay followed a fractional-conversion model. Thermal texture loss has been attributed to beta-eliminative depolymerisation and solubilisation of pectin and consequently loss of structural integrity. This was confirmed by microscopic and molecular observations where respectively cell separation and a pronounced increase in WSP were observed. In case of HP/HT processing, the thermal texture degradation was slowed down. At rather high-temperatures (95 – 110 °C), hardness also decayed following a fractional-conversion model but the degradation was a tenfold slower as compared to 0.1 MPa. In case of lower temperatures (80 °C), carrot hardness even remained constant (after an initial loss due to turgor loss). At microscopic and molecular levels, HP/HT processed carrot tissue was characterized by limited cell separation and almost no pectin solubilisation. The observations clearly indicated an inhibition of the beta-elimination reaction by pressure. The latter could be due to a direct and/or indirect effect of the elevated pressure. Direct inhibition is possible if the reaction is accompanied by an increase in reaction volume. However, seen the remarkably lower pectin DM of the HP/HT treated carrots, indirect inhibition of the beta-elimination (due to pronounced demethoxylation) seems more likely. The latter was corroborated by the results obtained on pectin model systems. At 0.1 MPa and elevated temperature, beta-eliminative depolymerisation (and chemical demethoxylation) occurred at a significant rate. When combining elevated temperature and pressure the rate of beta-elimination became zero whereas there was a pronounced stimulation of the demethoxylation. The pronounced demethoxylation during the HP/HT process was probably also responsible for the observation that a HP pretreatment had no additional effect on the subsequent carrot texture degradation. Texture degradation could remarkably be additionally reduced by simply replacing the treatment medium water by a Ca^{2+} solution.

When comparing equivalent thermal and HP processes, HPP proved to be preferred regarding texture preservation, both for pasteurization and sterilization intensities. In case of HP pasteurization, the processing temperature was too low

to induce beta-elimination which could explain the limited texture loss. In case of HP sterilization, the smaller texture loss as compared to thermal sterilization could be attributed to faster heating and cooling and correspondingly shorter processing time. In this respect, a HP/HT process can be seen as a high-temperature short-time process for slow (conduction) heating foods. However, the benefit of applying HP goes further. As proven in this work, the elevated pressure can inhibit or stimulate (depending on the positive or negative volume change) reactions occurring in plant tissues, thereby influencing particular quality characteristics. By stimulating pectin demethoxylation, the added HP is responsible for an inhibited beta-elimination and consequently better texture preservation.

This study was limited to the effect of HP/HT processing on the texture of carrots. Although by using carrots and pectin model systems generic insight was obtained, other plant families and/or varieties might react differently due to diverse biological differences, e.g. low pectin content, other pectin composition, differences in endogenous enzymes, presence of other texture governing factors/components. A more holistic picture could be obtained if more case studies would be included. Next to texture, other important nutritional and sensory quality characteristics also suffer from extensive heat treatments. Due to the shorter processing time, they are also expected to benefit from combined HP/HT processing. However, pressure might have an additional (positive or negative) effect by influencing related (bio)chemical reactions. As a consequence, evaluating the possible effect of HP/HT processing is not that straightforward and should be studied for each quality attribute of interest. Until now, it has not been univocally demonstrated whether the elevated pressure acts synergistically with HT for spore inactivation. If that would be the case, lower processing temperatures could be applied. This would result in less impact on heat-labile quality characteristics. In this context, the consumer may not be neglected. Overall, his/her demand for fresh-like products is driving this research and in the end he/she will decide whether he/she buys the product or not. Therefore, it is important to study consumer attitudes towards this technology and towards HP/HT processed food products. Likewise, sensory evaluation studies of these products are indispensable. It can be concluded that, before commercial introduction of HP sterilization, more research concerning the effect of combined HP/HT processing on food quality and safety and on consumer acceptance is needed. The currently running EU-funded Integrated Project "NovelQ" is dealing with several of the above-mentioned research questions.

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